Resistance of *Aedes aegypti* from Three Provinces in Indonesia, to Pyrethroid and Organophosphate Insecticides

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Abstract

Resistance of Aedes aegypti to insecticides has become a threat in the effort to control the vector for DF/DHF virus. In this research, Ae. aegypti *collected from three cities in Indonesia, i.e. Bandung, Palembang, and Surabaya, were tested to determine their resistance status to pyrethroid (permethrin and deltamethrin) and organophosphate (temephos) insecticides. The resistance level was expressed as resistance ratio (RR) compared to the susceptible VCRU strain. Result of this study showed that resistance level of Ae. aegypti in Indonesia to permethrin, deltamethrin, and temephos is still low, except for Bandung strain to permethrin and deltamethrin. However, all strains have shown an increase tolerance to all insecticides tested compared to VCRU strain, suggesting that if the pesticides are used continuously, resistance can develop and it can be a major problem. We expect that our finding could be used to help the health authority to design a better mosquito control program.*

Keywords : *Aedes aegypti, resistance, insecticides, pyrethroid, organophosphate*

I. Introduction

Ae. aegypti is a primary vector for Dengue Fever/Dengue Haemorrhagic Fever (DF/DHF) virus (16). The intensive and continuous use of insecticides to control this mosquito has caused the development of resistance of *Ae. aegypti* to many insecticides. Researches in many countries have found that *Ae. aegypti* were already resistant to all classes of insecticides. For example, *Ae. aegypti* in Thailand (10,19), Vietnam (7), Brazil (9), Cuba and Venezuela (11) have been resistant to organophosphate and pyrethroids insecticides. Resistance of *Ae. aegypti* to various classes of insecticides is also occured in Indonesia. For example, researches conducted by Arief (2000) and Butar Butar (1998) have shown that *Ae. aegypti* collected in Bandung and several other cities in West Java, Indonesia (Ciamis, Purwakarta, and Bogor) have been resistant to carbamate (propoxur) and pyrethroids (deltamethrin and permethrin) (1,2).

Resistance to insecticides can be a threat in the vector control program, because it causes certain insecticides to be ineffective to control mosquitoes. This may reduce the lifetime of an insecticide, and failure to control mosquitoes can cause a population explosion of the mosquitoes that lead to the explosion of the disease. However, in the meantime, available information about resistance status of mosquitoes in South East Asia is not yet adequate to determine a policy about effective insecticides use in mosquito control program (17).

In this research, resistance assay were conducted to determine resistance status of *Ae. aegypti* collected from three cities in Indonesia, i.e. Bandung, Palembang, and Surabaya, to two pyrethroid insecticides (permethrin and deltamethrin) and an organophosphorus insecticides (temephos). Pyrethroids are commonly used as active ingredient of commercial insecticides, while temephos is an active ingredient of Abate, larvicide commonly used in Indonesia.

Data about resistance status of *Ae. aegypti* is very important and can be used by the authorities to determine a mosquito control strategy, that is effective and does not encourage further resistance. The strategy is important in the effort to control the population of *Ae. aegypti* in long term, to prevent the development of resistance to insecticides as well as the possibility of DF/DHF case explotion in the future due to the failure of mosquito control program.

II. Materials

Mosquitoes. Three mosquito strains were tested in this research. They were collected from three cities in Indonesia, i.e. Bandung, Palembang, and Surabaya. Mosquito from Bandung were collected by placing ovitraps in people's houses, while mosquitoes from Surabaya and Palembang were obtained from Health Research and Development, Ministry of Health Jakarta. A pure susceptible VCRU strain (obtained from Vector Control Research Unit Universiti Sains Malaysia) was used as standard strain. All strains were reared in Laboratory of Entomology, School of Life Sciences and Technology, Institut Teknologi Bandung before use.

Insecticides. Insecticides used were permethrin 0,75%, deltamethrin 0,05%, and temephos. Permethrin and deltamethrin were purchased from WHO in the form of insecticide impregnated paper. Temephos used was as Abate 1SG.

III. Methods

Resistance assay. Assay to determine resistance status of *Ae. aegypti* to permethrin and deltamethrin was conducted using method from WHO. A set of adult mosquitoes (2-5 days old) were exposed to insecticides for certain period of time (5, 10, 15, 30, 45, and 60 minutes). The mosquitoes then were allowed to recover and after 24 hours the number of dead mosquitoes was counted. The result was analyzed using probit analysis to determine LT_{50} and LT_{90}. The resistance level was determined by comparing the LT values of mosquito tested to the LT values of VCRU strain.

Assay to determine resistance status of *Ae. aegypti* to temephos was conducted using method from WHO. Fourth instar of *Ae. aegypti* larvae were exposed to a series of temephos with different concentrations. After 24 hours exposure, the number of dead larvae was counted and was analyzed using probit analysis to determine LC_{50} and LC_{95}. The resistance level was determined by comparing the LC values of mosquito tested to the LC values of VCRU strain.

IV. Results and Discussion

Results of the assay showed that all strains tested were already developed resistance to insecticides tested. Bandung strain was the most resistant strain. It was resistant to permethrin and deltamethrin with RR_{90} 79.3 and 23.7 respectively (table 1 and 2). Palembang strain was slightly resistant to permethrin (RR_{90} 11.1) but still susceptible to deltamethrin (RR_{90} 2.2). Surabaya 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) that a population is considered resistance if RR is more than 10 (8). However, although Surabaya strain was considered susceptible to permethrin and deltamethrin and Palembang strain was considered susceptible to deltamethrin, the increased RR compared to VCRU strain suggested that those strains were already developed tolerance to permethrin and deltamethrin, and that they may develop resistance in the future.

Strain	n	LT_{50} (min.)	RR_{50}	LT_{90} (min.)	RR_{90}	Slope ± SD
VCRU	380	2.3	1	12.8	1	1.724 ± 0.315
PAL	425	11.3	4.9	141.8	11.1	1.166 ± 0.168
SBY	416	13.5	5.9	109.9	8.6	1.409 ± 0.179
BDG	399	105.2	45.7	1015.1	79.3	1.302 ± 0.223

Table 1. Resistance level of *Ae. aegypti* to permethrin
VCRU (Vector Control Research Unit, a susceptible pure strain of *Ae. aegypti*), PAL (Palembang), SBY (Surabaya), BDG (Bandung), RR (Resistance Ratio), LT_{50} (Lethal Time 50%), LT_{90} (Lethal Time 90%), n (number of mosquito tested), SD (Standard Deviation)

Assay on mosquito larvae showed that all strains tested were already developed tolerance to temephos. All strain showed slight increase in LC_{95} value compared to VCRU strain (table 3). This indicated that although Bandung, Surabaya, and Palembang strain were still susceptible to temephos, there was a portion of the population that has developed resistance to temephos. This was also indicated by the slope of those strains that was lower than VCRU strain (table 3), suggesting that those strains were more heterogenous than VCRU strain. The result is not surprising, since temephos has been widely used in Indonesia as larvicide to control mosquito larvae.

Resistance of Bandung 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 (13). The α–cyano gives steric effect on deltamethrin that causes deltamethrin to be more stable and difficult to be hydrolyzed on its ester bond (13,14,15).

Cross resistance to several types of pyrethroid insecticides have been found in many mosquito species from many countries, among others are *Anopheles gambiae* from Cote d'Ivoire (2) and *An. stephensi* and *An. culicifacies* from India (6). However, there was no indication of cross resistance between pyrethroids and organophosphate insecticides in Bandung strain, because although it had high level of resistance to pyrethroids, it was still susceptible to temephos (organophosphate). There were also no indication of cross resistance in other strains, because of the low levels of resistance in those strains to pyrethroids as well as temephos.

Strain	**n**	**LT_{50} (min.)**	**RR_{50}**	**LT_{90} (min.)**	**RR_{90}**	**Slope ± SD**
VCRU	461	2.0	1.0	22.1	1.0	1.236 ± 0.212
PAL	437	2.1	1.05	48.6	2.2	0.948 ± 0.191
SBY	447	4.2	2.1	55.8	2.5	1.142 ± 0.183
BDG	429	25.5	12.75	524.5	23.7	0.976 ± 0.171

Table 2. Resistance level of *Ae. aegypti* to deltamethrin
VCRU (Vector Control Research Unit, a susceptible pure strain of *Ae. aegypti*), PAL (Palembang), SBY (Surabaya), BDG (Bandung), RR (Resistance Ratio), LT_{50} (Lethal Time 50%), LT_{90} (Lethal Time 90%), n (number of mosquito tested), SD (Standard Deviation)

Strain	**LC_{50}(mg/L)**	**LC_{95} (mg/L)**	**Slope**	**RR_{50}**	**RR_{95}**
VCRU	0.0026	0.0056	4.965	1.0	1.0
Surabaya	0.0079	0.0244	3.357	3.0502	4.3964
Palembang	0.0040	0.0106	3.863	1.5290	1.9027
Bandung (ITB)	0.0035	0.0101	3.610	1.3629	1.8144
Bandung (Ujung Berung)	0.0021	0.0133	2.071	0.8263	2.3982
Bandung (Surapati)	0.0024	0.0068	3.548	0.9073	1.2324

Table 3. Resistance level of *Ae. aegypti* larvae to temephos
VCRU (Vector Control Research Unit, a susceptible pure strain of *Ae. aegypti*), RR (Resistance Ratio), LC_{50} (Lethal Concentration 50%), LT_{90} (Lethal Concentration 90%),
n (number of mosquito tested)

Resistance of Bandung strain to permethrin and deltamethrin may be due partly to the increase 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 (4,5,9,12,18,19).

A related study in our Lab. (unpublished) showed that activity levels of detoxifying enzymes (oxidases, esterase A, and esterase B) were significantly higher in Bandung strain compared to the susceptible VCRU strain (data not shown), suggesting that those enzymes might be involved in the development of resistance in Bandung strain. The high level of resistance in Bandung strain presumably was caused by the cooperation of the enzymes in detoxifying permethrin and deltamethrin entering insects body. Esterases detoxify pyrethroids through hydrolysis of ester bond, while oxidases detoxify pyrethroids through hydroxylation (adding –OH) or oxidation of ester bond of pyrethroids (13).

The involvement of oxidases and esterases in resistance to pyrethroids may cause cross resistance to organophosphorus insecticides since they can detoxify pyrethroids as well as organophosphorus insecticides. The result of this study indicated that all strain tested were still susceptible to temephos. However, the high activity level of oxidases, esterase A, and esterase B that can be related to the high level of resistance to pyrethroids in Bandung strain may cause resistance to organophosphate in the future. Therefore, continuous monitoring of resistance level of mosquito to both classes of insecticides is very important and must be done to prevent the development of resistance in the future.

V. Acknowledgements

This work was partly supported by a competitive grant from the Directorate General of Higher Education Republic of Indonesia ,and Institut Teknologi Bandung given to Intan Ahmad.

VI. References

1. R. Arief, Skripsi Sarjana, Departemen Biologi Institut Teknologi Bandung (2000)
2. E.N.T. Butar Butar, Skripsi Sarjana, Departemen Biologi Institut Teknologi Bandung (1998)
3. F. Chandre, F. Darriet, S. Manguin, C. Brengues, P. Carnevale, P. Guillet, J. Am. Mosq. Control Assoc. 15, 1 (1999)
4. T. Chareonviriyaphap, P. Rongnoparut, P. Chantarumporn, M.J. Bangs, Journal of Vector Ecology. 28, 1 (2003)
5. J. Etang, F. Chandre, P. Guillet, L. Manga, Malaria Journal. 3, 46 (2004)
6. K.N. Ganesh, J. Urmila, V.A. Vijayan, Indian Journal of Medical Research. (2003)
7. V.D. Huong, N.T.B. Ngoc, Dengue Bulletin Volume 23 (1999)
8. H. Liu, E.W. Cupp, A. Gui, N. Liu, J. Med. Entomol. 41, 5 (2004)
9. M.L.G. Macoris, M.T.M. Andrighetti, L. Takaku, C.M. Glasser, V.C. Garbeloto, J.E. Bracco, Mem Inst Oswaldo Cruz. 98, 5 2003)
10. A. Ponlawat, J.G. Scott, L.C. Harrington, J. Med. Entomol. 42, 5 (2005)
11. M.M. Rodriguez, J. Bisset, D.M. De Fernandez, L. Lauza, A. Soca,) J. Med. Entomol. 38, 5 (2001
12. M.M. Rodriguez, B. Bisset, M. Ruiz, A. Soca, J. Med. Entomol. 39, 6 (2002)
13. G.A. Kerkut and L.I. Gilbert (Eds), Comprehensive Insect Physiology Biochemistry and Pharmacology Vol. 12: Insect Control, Pergamon Press, Oxford, (1985)
14. H. Vatandoost, Acta Medica Iranica. 42, 2 (2004)
15. C.E. Wheelock, J.L. Miller, M.J. Miller, S.J. Gee, G. Shan, B.D. Hammock, Environmental Toxicology and Chemistry. 23, 11 (2004)
16. WHO SEARO, Prevention and Control of Dengue and Dengue Haemorrhagic Fever: Comprehensive Guidelines (Online) (2004)
17. WHOSEA, (Online) (http://w3.whosea.org/EN/section10/section332.htm) (2004)
18. J.M. Vulule, R.F. Beach, F.K. Atieli, J.C. McAllister, W.G. Brogdon, J.M. Roberts, R.W. Mwangi, W.A. Hawley, Medical and Veterinary Entomology. 13, 3 (1999)
19. R. Yaicharoen, R. Kiatfuengfoo, T. Chareonviriyaphap, P. Rongnoparut, Journal of Vector Ecology. 30, 1 (2005)