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Variation in susceptibility status to organophosphate insecticide among several geographic populations of *aedes albopictus* skuse in Indonesia

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ABSTRACT

Budi Mulyaningsih – ***Variation in susceptibility status to organophosphate insecticide among several geographic populations of Aedes albopictus Skuse in Indonesia***

Background: *Aedes albopictus* skuse is an important vector of dengue fever and dengue hemorrhagic fever in Southeast Asia. Its distribution in Indonesia extends from Sabang (Sumatera) to Merauke (Papua) and is currently expanding. In response to a potential dengue fever outbreak in Indonesia during 2002, the susceptibility of 4 geographic populations of *Aedes albopictus* Skuse to commonly used mosquito adulticide (malathion) and larvicide (temephos) were assessed.
Objective: The aim of this study is to determine the susceptibility status of each *Ae. albopictus* population to malathion and temephos.
Methods: Eggs collected from 4 different geographic area in Indonesia, such as Padang (Sumatera), Yogyakarta (Java), Banjar (Kalimantan) and Timika (Papua) were reared to larvae and adults and were investigated by using bioassay (World Health Organization, 1981). The bioassay data were analyses using Probit analysis, which also corrects for mortality in the controls by Abott formula.
Result: The *Ae. albopictus* mosquito population originate from Yogyakarta shows highest LC_{50} and LC_{90} malathion and temephos value, and the *Ae. albopictus* mosquito population originate from Timika shows lowest LC_{50} and LC_{90} malathion and temephos value than other population.
Conclusion: There were insecticide susceptibility status difference of *Ae. albopictus* from different geographic population in Indonesia (Padang, Yogyakarta, Banjar and Timika).

Key words: malathion – temephos - *Ae. albopictus* - bioassay - Abott formula.

ABSTRAK

Budi Mulyaningsih – ***Variasi status kerentanan nyamuk Aedes albopictus Skuse dari beberapa populasi geografis di Indonesia terhadap insektisida organofosfat.***

Latar belakang: *Aedes albopictus* skuse merupakan vektor demam berdarah dengue yang penting di Asia Tenggara. Di Indonesia penyebarannya sangat luas dari Sabang (Sumatera) sampai ke Merauke (Papua). Untuk meningkatkan usaha pengendalian vektor DBD maka perlu diteliti status kerentanan nyamuk *Ae. albopictus* dari beberapa populasi geografis di Indonesia terhadap insektisida organofosfat.
Tujuan: Menentukan status kerentanan nyamuk *Ae. albopictus* dari beberapa populasi geografis di Indonesia terhadap insektisida organofosfat.
Bahan dan cara: Telur nyamuk yang diperoleh dari beberapa daerah dengan kondisi geografis yang berbeda di Indonesia, yaitu Padang (Sumatera), Yogyakarta (Jawa), Banjar (Kalimantan) dan Timika (Papua) dikolonisasi sampai menjadi larva dan nyamuk dewasa untuk selanjutnya diuji dengan bioasai (World Health Organization, 1981). Data yang diperoleh dianalisis menggunakan Probit, dan kematian nyamuk kontrol dikoreksi dengan formula Abott.

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Hasil: Nyamuk *Ae. albopictus* yang berasal dari Yogyakarta menunjukkan nilai LC_{50} dan LC_{90} malation dan temefos yang tertinggi dan nyamuk *Ae. albopictus* yang berasal dari Timika menunjukkan nilai LC_{50} dan LC_{90} malation and temefos yang terendah dibanding dengan populasi lain.
Simpulan: Status kerentanan nyamuk *Ae. albopictus* yang bersal dari Padang (Sumatera), Yogyakarta (Jawa), Banjar (Kalimantan) dan Timika (Papua) menunjukkan perbedaan yang nyata.

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INTRODUCTION

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are serious diseases of Asia and Africa, and in Indonesia they become major public health problems. The first out break of dengue occurred in Surabaya and Jakarta in 1968. Since then the reported cases and the number of provinces and districts affected have gradually been increasing, and until now there are 288 districts among 310 (93%) in all provinces.[1]

Dengue is spread by two species of the *Aedes* mosquito. The main vector of dengue is *Aedes aegypti* Linnaeus (*Ae. aegypti*) and a close relative, *Aedes albopictus* Skuse (*Ae. albopictus*) which is also involved in dengue transmission as a secondary vector. Although *Ae. albopictus* is of lesser importance in the transmission of dengue, studies indicate that it is more susceptible to infection than *Ae. aegypti* and, as it can tolerates cooler conditions, it is now more important. Its wider range, modern air and surface transport has hastened its distribution. Because of these condition *Ae. albopictus* is among the most important arbovirus in the world, particularly for dengue virus.[2,3]

Aedes albopictus is presumed to have originated in Southeast Asia, also the indigenous home of the dengue viruses, and also considered to be the original vector of dengue[4]. In Indonesia its distribution extends from Sabang (Sumatera) to Merauke (Papua) and has currently been expanding. Geographical condition of Indonesia which shows varying climatology, biography and environmental factors seem to be quite favorable for survival and the continuation of life cycle of various species of mosquitoes and their bionomics.

Upto this moment, the prevention and constrain of DHF only depends on the success of the vector control which is the *Aedes aegypti* and *Aedes albopictus* by using insecticides.[5] Potencies of mosquitoes vectorizing and insecticide susceptibility could differ either interspecifically or intraspecifically among different geographical areas[6]. That is why health problem due to mosquito borne disease could be local area spesific.

In Indonesia, two organophosphate insecticides, such as malathion and themephos have been commonly used for controlling *Aedes* mosquito to stop DHF transmission since 1970.[7] The long practice of applying such chemicals on the target insect could be one possible factor related to the development of resistance in the dengue vector. To help the Indonesian Department of Health combating the spread of dengue fever in Indonesia, susceptibility of *Ae. albopictus* toward organophosphate insecticide (malathion and temephos) were evaluated. It is expected that the following results would be useful to improve the control of DHF vector, particularly in rural area, where *Ae. albopictus* were dominan.

MATERIALS AND METHODS

Ae. albopictus mosquitoes were collected from 4 different geographic area in Indonesia such as Padang (Sumatera), Yogyakarta (Java), Banjar (Kalimantan) and Timika (Papua) through ovitrap surveys. After hatching, the larvae were colonized in the laboratory until the adults emerged and the species confirmed (differentiated). Colonization of the mosquitoes were continued to obtain the F1 generations of larvae and adult stages, and they were subjected for resistance tests. Dilutions were prepared from technical grade solutions for each insecticide.

Malathion impregnated papers were prepared by spreading 5 ml of the required insecticide/ acetone solution on 12 x 15 cm rectangles of Whatman's no 1 filter paper. The malathion impregnated papers were prepared for each of the following concentration (µg/5ml/paper): Padang (0.30; 0.60; 1.20; 2.40; 4.80; 9.60 and 19.20), Yogyakarta (0.50; 1.00; 2.00; 4.00; 8.00; 16.00 and 32.00), Banjar (0.15; 0.30; 0.60; 1.20; 2.40; 4.80 and 9.60) and Timika (0.05; 0.10; 0.20; 0.40; 0.80; 1.60 and 3.20).

For the second instar larvae test, temephos was prepared for each of the following concentration (ppm): Padang (0.010; 0.020; 0.040; 0.080; 0.160; 0.320), Yogyakarta (0.040; 0.080; 0.160; 0.320; 0.640 and 1.280), Banjar (0.010; 0.020; 0.040; 0.080 and 0.160) and Timika (0.005; 0.010; 0.020; 0.040; 0.080 and 0.160). For early fourth instar larvae test, temephos was prepared for each of the following concentration (ppm): Padang (0.100; 0.150; 0.200; 0.250; 0.300; 0.350 and 0.400), Yogyakarta (0.010; 0.020; 0.040; 0.080; 0.160; 0.320 and 0.640) and Timika (0.005; 0.010; 0.020; 0.040; 0.080; 0.160 and 0.320).

Kits and procedures produced by the World Health Organization were used for testing the susceptibility of larval and adult mosquitoes with some modifications[8]. Second and early fourth instar larvae were exposed to several concentrations of the temephos test solutions. Adults female mosquito were exposed to several impregnated papers of the malathion test solutions. Groups of approximately thirty larvae or adult mosquitoes per test were employed in five or more concentrations of each insecticide and at least in five replications on different days. Mosquitoes mortality were scored at the end of a 24 hours holding period. Results were subjected to probit analysis by the method of Finney[9], and LC_{50}, LC_{90}, fiducial limit and slopes values were obtained.

RESULTS

The results of susceptibility test with malathion and temephos for *Ae. albopictus* mosquitoes population (adult and larvae) collected from several different geographic areas in Indonesia (Padang, Yogyakarta, Banjar and Timika) showed different susceptibility status. Log-dose probit mortality data for adult *Ae. albopictus* tested with malathion are presented in TABLE 1.

TABLE 1. Susceptibility status to malathion of *Ae. albopictus* from several different geographic areas in Indonesia

Ae. albopictus population	LC_{50}	95% conf. interval	LC_{90}	95% conf. interval	Slope	SE
Padang	3.375	3.004-3.809	19.630	13.817-21.548	1.676	0.075
Yogyakarta	5.287	4.694-5.930	27.125	20.650-30.893	1.911	0.098
Banjar	1.309	1.167-1.468	6.620	5.403-8.110	1.846	0.094
Timika	0.503	0.462-0.613	2.674	2.130-3.356	1.850	0.101

LC_{50} and LC_{90} values in µg/5ml/paper

The results (TABLE 1) shows that *Ae. albopictus* mosquito population originated from Yogyakarta have higher LC_{50} and LC_{90}, and *Ae. albopictus* mosquito population originated from Timika have lower LC_{50} and LC_{90} than the *Ae. albopictus* mosquito population originated from other areas when treated with malathion. In other words *Ae. albopictus* mosquito population originated from Yogyakarta are more resistant and *Ae. albopictus* mosquito population originated from Timika are more susceptible to malathion. Log-dose Probit mortality data for larva-2 *Ae. albopictus* tested with temephos are presented in TABLE 2.

The *Ae. albopictus* larva-2 population originated from Yogyakarta have higher LC_{50} and LC_{90}, and *Ae. albopictus* (larvae-2) population originated from Timika have lower LC_{50} and LC_{90} than other population when treated with temephos (TABLE 2). Log-dose probit mortality data for

TABLE 2. Susceptibility status to temephos of *Ae. albopictus* larvae-2 from several different geographic areas in Indonesia

Ae. albopictus larvae-2 population	LC_{50}	95% conf. interval	LC_{90}	95% conf. interval	Slope	SE
Padang	0.066	0.059-0.074	0.291	0.237-0.357	2.024	1.149
Yogyakarta	0.240	0.215-0.268	0.724	0.652-0.804	2.081	0.117
Banjar	0.041	0.037-0.045	0.147	0.121-0.180	2.434	0.145
Timika	0.035	0.035-0.031	0.138	0.136-0.114	2.090	0.116

LC_{50} and LC_{90} values in ppm

larva-4 *Ae. albopictus* tested with temephos are presented in TABLE 3. The *Ae. albopictus* larvae-4 originated from Yogyakarta shows higher LC_{50} and LC_{90}, and the *Ae. albopictus* larvae-4 originated from Timika shows lower LC_{50} and LC_{90} than other population when treated with temephos (TABLE 3). In other words *Ae. albopictus* (larva-2 and larva-4) population originated from Yogyakarta are more resistant and *Ae. albopictus* (larva-2 and larva-4) population originated from Timika are more susceptible to temephos.

The susceptibility status difference between the *Ae. albopictus* mosquito population originated from Padang, Yogyakarta, Banjar and Timika also can be detected by fiducial limits and the slopes of the line. The results of this study shows that the range of the LC_{50} and LC_{90} (malathion and temephos) are not wide and not overlap. This situation indicates that the susceptibility status of

TABLE 3. Susceptibility status to temephos of *Ae. albopictus* larvae-4 from several different geographic areas in Indonesia

Ae. albopictus larvae-2 population	LC_{50}	95% conf. interval	LC_{90}	95% conf. interval	Slope	SE
Padang	0.066	0.059-0.074	0.291	0.237-0.357	2.024	1.149
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LC_{50} and LC_{90} values in ppm

Ae. albopictus mosquito from each population are extremely different. The slope of the line for the *Ae. albopictus* mosquito population originated from Banjar are more vertical than from other population. It means that the response of individual *Ae. albopictus* to malathion and temephos in Banjar population are more homogen than other population.

DISCUSSION

Malathion as adulticide and temephos as larvicide have been mainstay of dengue vector control in Indonesia for more than 27 years. According to the Ministry of Health of Indonesia, Yogyakarta, Padang and Banjar are categorized as dengue endemic areas and Timika is non endemic dengue area.[7]

This study shows that bioassay result of *Ae. albopictus* mosquitoes (adult and larvae) from each population in Indonesia (Padang, Yogyakarta, Banjar and Timika) using several concentrations of malathion and themephos under laboratory conditions shows different susceptibility status to the two insecticides. Dharmawan[10], suggested that response of a mosquito species in an area to a particular insecticide may not be the same with area.

The genetic factor that influences the response of each mosquito population to the insecticide is the resistant gene (R-gene), arranges the occurrence of resistance on each *Ae. albopictus* population.

French-Constant & Bonning reported that one of the mechanisms of an insect's resistance to organophosphate insecticide is the increasing of esterase enzyme activity.[11] The increase of esterase enzyme activity reduces the lethal dose effect of insecticide againts targeted insect. A pattern of the esterase enzyme from each mosquito species, whereby it is either specific or unique, making it applicable as a tool to differentiate species, is an indicator of the presence of geo-graphical variation[12, 13, 14].

Such evidence as obtained by the biochemical assay showed some heterogenities of *Ae. albopictus* in Indonesia, and showed the presence of genotypic polymorphism in the mosquito population possibly influencing the mechanism of resistance to the insecticides.[15]

CONCLUSION

The geographical variation might influence the susceptibility status of each *Ae. albopictus* mosquito population to the insecticide, namely malathion and temephos.

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