

SUSCEPTIBILITY OF DENGUE HAEMORRHAGIC FEVER VECTOR (*Aedes aegypti*) AGAINST ORGANOPHOSPHATE INSECTICIDES (MALATHION AND TEMEPHOS) IN SOME DISTRICTS OF YOGYAKARTA AND CENTRAL JAVA PROVINCES

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Abstrak. Suatu penelitian untuk mengetahui suseptibilitas vektor DBD *Aedes aegypti* terhadap insektisida organofosfat (malathion dan temefos), di beberapa daerah endemis DBD di Provinsi Yogyakarta dan Jawa Tengah, telah dilakukan di Balai Penelitian Vektor dan Reservoir Penyakit, Salatiga, bulan Juni 2005. Sampel jentik diambil dari tempat penampungan air habitat *Ae. aegypti* di dalam rumah penduduk di daerah endemis DBD seperti: kota Yogyakarta, Surakarta, Semarang dan Salatiga. Penelitian dilakukan secara biokimia dengan uji esterase enzym non spesifik dan uji suseptibilitas menggunakan impregnated paper (malathion 0,5 dan 0,8%), metode WHO (1975). Hasil penelitian menunjukkan bahwa status populasi vektor DBD dari kota Yogyakarta 20,83% toleran, 35,41-58,33% resisten, Surakarta 25,0-29,16% toleran, 37,50-45,83% resisten, Semarang 29,16-37,50% toleran dan 33,33%-62,50% resisten, sedangkan dari Salatiga 41,70% toleran dan 16,60% resisten terhadap insektisida malathion. Populasi nyamuk *Ae. aegypti* dari semua daerah penelitian terbukti masih susptibel terhadap temefos (Abate 1% SG) dengan kematian 99-100%.

Key words: vektor; demam berdarah dengue; resistens; Jateng-DIY.

INTRODUCTION

The research held in Yogyakarta province, Indonesia⁽¹⁾, had reported the reduction of susceptibility of dengue vector *Aedes aegypti* larvae, against organophosphate (temephos and malathion), after such a long term usage (more than 20 years). In addition, this condition caused an increase on resistance status, which was correlated with the high activity of non-specific esterase enzyme on mosquito larvae. Furthermore, the non-specific esterase enzyme would have hydrolyzed the α -naphthyl acetate substrate on *Aedes aegypti*' body.

The organophosphate insecticide such as temephos and malathion had been used since 1976, for larvae and mosquito control, respectively. The effectiveness of these insecticides are never been evaluated,

after more than 20 years' usage. Related to this situation, it was suspected that the frequent used of organophosphate insecticides especially temephos and malathion in some areas of Central Java, could cause an increase of resistance status on dengue vector. Several methods can be used to evaluate the resistance status of mosquito vectors against insecticides. Biochemical test is a more applicable method in this application (which correlates with non-specific esterase enzymes elevated activity mechanism compared to conventional method the susceptibility and bioassay tests⁽²⁾.

The objective of this study is to evaluate the susceptibility of DHF vector *Ae. aegypti* against insecticide organophosphate compound (temephos and malathion)

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after more than 20 years 'usage (3 times application each year).

II. MATERIALS AND METHODS

Aedes aegypti larvae (instar IV) were collected from the DHF endemic areas of Yogyakarta and Central Java Provinces. The study was conducted in some districts of Yogyakarta (Yogyakarta Province) and Surakarta, Semarang and Salatiga (Central Java Province), in June to October 2005 in the Vector and Reservoir Control Research Unit, Salatiga.

A descriptive cross sectional research design was applied in the study, which was a non-experimental research design. Study was also conducted to determine correlation between the period of insecticide usage (temephos and malathion) and the reduction of susceptibility of *Ae. aegypti* larvae, against Organophosphat insecticide (especially malathion and temephos). Biochemical analysis, was done to evaluate the susceptibility status of *Ae. aegypti* larvae against Organophosphate insecticide (malathion and temephos).

Test of non-specific esterase enzyme, based on Lee's method (1990)

Aedes aegypti larvae (instar IV) were homogenate individually and diluted with 0.5 ml phosphate buffer saline (PBS) 0.02 M solution, pH=7. Fifty microliters of homogenate was removed to microplates using micropipete. Substrate α -naftyl acetate in acetone (6 g/l) and 50 ml buffer phosphate (0.02 M; pH=7) volume 50 μ l, were added, and kept for about 60 second. In addition, add 50 μ l coupling reagent in a form of Fast Blue B (150 mg), namely (o-dianisidine, tetrazotized, sigma) in 15 ml aquadest and 35 ml aqueous (5%;w/v) sodium dodecyl sulphate (sigma). Wait for 10 minutes, until the red colour turns into blue. Stop the reaction by adding 50 μ l

acetate acid 10% into the microplates which was filled with homogenate. Colour intensity showed the non specific enzyme esterase activity and will be distinguished visually. Non-specific enzyme esterase will be quantitatively read by ELISA reader on the wavelength of (λ) 450 nm^(3, 4, 5).

Data interpretation. Biochemical test data appeared as colour intensity which resulted from the activity of non specific enzyme esterase. This enzyme are qualitatively described as colour score, according to empiric criteria⁽¹⁾; score < 2.0 (color none) = high susceptible (SS); 2.0-2.5 (light blue) = medium resistance (RS) and 2.6-3.0 (dark blue) = high resistance (RR)^(6, 7).

The data on biochemical test based on the color intensity which resulted from the activity of non specific esterase. The results will be quantitatively measured by the absorbance value (AV) using ELISA reader on λ = 450 nm. AV value < 0.700 (very susceptible=SS); AV = 0.700-0.900 (medium resistance=RS); AV > 0.900 (high resistance=RR).

Aedes aegypti susceptibility test against Malathion 0.5% and 0.8%^(7, 8, 9).

Tested mosquitoes (*Aedes aegypti*) were taken from the collected larvae in the study areas. Female mosquitoes from the colony (ages 3-4 days), were placed in plastic glasses (filled with 25 mosquitoes), and provided by wet cotton sugar cane (10%).

On the next day, all the tested mosquitoes were moved to the WHO susceptibility test kits (green marked) and on the other test kits (red marked), layered by Malathion impregnated paper, standard concentration 0.5% and 0.8%.

The tested mosquitoes in the green marked test kits were blown to the testing

test kits (red marked). It was done after 60 minutes exposure.

The knock-down and living mosquitoes were counted and moved to clean plastic cup (provided by cotton wet sugar cane), kept for 24 hours in room condition. The mosquito mortalities were counted after 24 hours holding (after the exposure)

Data Interpretation. According to WHO standard ⁽²⁾, the populations of tested mosquitoes are susceptible, if the mortality occurs as 98-100%. In addition, when the mortality is 80-98%, the mosquitoes are tolerant against insecticide and resistant when the mortality less than 80%.

Bioassay test of *Aedes aegypti* larvae against Abate (temephos 1% SG) ⁽⁸⁾.

First, dilute 2 gram of Abate (temephos 1% SG) in 20-liter water. Second, provide 24 plastic bowls (volume: 1 liter), each filled with (500 ml volume) temephos (2g/20 lt water). Third, each bowl was filled with 25 *Aedes aegypti* larvae (concentration 1 g/10lt) with 4 times replication for every location and 1 time replication for the control treatment (UTC= untreated control). Fourth, an observation of tested larvae mortality was done 24-hour after application. The results were formatted on percentage for every sample location (24 hour after treatment)

Data analysis: Bioassay test result was analyzed descriptively.

IV. RESULTS AND DISCUSSION

Bioassay test/Esterase (%) samples from Yogyakarta municipality, DIY province.

Forty-eight individual samples of *Ae. aegypti* larvae were collected from Wirobrajan sub district, showed that 20.83% of larvae were still susceptible, 20.83% tolerant and the rest 58.33% had been resistant

against Organophosphate insecticides. This condition caused by the elevated activity of esterase enzyme in their bodies. On the other hand, a different result from Tegalrejo sub district showed that 48 individual samples, 43.75% were susceptible, 20.83% tolerant and 35.41% resistant, against organophosphate.

The susceptibility test of *Ae. aegypti* mosquitoes were collected from Wirobrajan sub district. In addition, (using malathion impregnated paper) by using 0.5% and 0.8% concentrations, the result revealed that 8.0% and 36.0% mortality occurred respectively. The results were taken from 100 tested mosquitoes samples, which were collected from the treated study area.

Samples which were collected from Tegalrejo subdistrict showed that on 0.5% concentration of malathion impregnated paper 10.0% mortality occurred. While on 0.8% concentration (by using malathion impregnated paper), 52.0% mortality occurred. These results indicated that those species was resistant against malathion (less than 80% mortality is considered to be resistant; according to WHO standard).

The bioassay test of *Ae. aegypti* larvae against Abate (Temephos 1% SG) showed that the population taken from Wirobrajan and Tegalrejo subdistricts were still susceptible against Temephos 1% SG (1 g/10 liters water) revealed 99-100% mortality.

Bioassay test/Esterase sample collected from Surakarta, Central Java province.

Samples collected from Jebres sub district (48 individual samples) showed that 25.00% *Aedes aegypti* larvae were still susceptible, 29.16% tolerant and the rest (45.83%) had been resistant against Organophosphate insecticides. On the other hand, a different result on Kampung

Sewu sub district showed that 37.50% were susceptible, 25.00% were tolerant and 37.50% were resistant againts organophosphate (Figure 1: Attachment 1).

The susceptibility test on *Ae. aegypti* larvae with malathion which was done into the samples collected from Jebres subdistrict, showed no mortality (0.00%) in 0,5% impregnated paper concentration. On the other hand, a 58.0% mortality was found after using 0.8% concentration of impregnated paper (using 100 tested mosquitoes).

Samples which were collected from Kampung Sewu sub district (using 100 tested mosquitoes) showed the results as follow : no mortality occurred (0.00%) on 0.5% impregnated paper concentration, but 63.00% mortality occurred on the use of 0.8% concentration. These results indicated that *Ae. aegypti* population from that area were resistant againts Malathion) (Figure 2; Attachment 1).

The bioassay test on *Ae. aefypti* larvae against Abate (temephos 1% SG, concentration 1 g/10 liters water) which was done into the samples collected from

Jebres and Kampung Sewu subdistrict, showed 100% mortality (Figure 3, Attachment 1).

Bioassay test/Esterase samples collected from Semarang, Central Java province

Samples collected from Kalipancur sub district (48 individual samples) showed that 8.34% *Aedes aegypti* larvae were still susceptible, 29.16% tolerant and the rest (62.50%) had been resistant against organophosphate insecticides. However, a different result on Klipang sub district showed that 29.16% were susceptible, 37.50% were tolerant and 33.33% were resistant against organophosphate.

The results of susceptibility test of *Ae. aegypti* mosquitoes which was done into samples collected from Kalipancur sub district (using malathion) showed that on 0.5% concentration had caused 12.00% mortality, while on 0.8% concentration had caused 65.00% mortality on 100 tested mosquitoes.

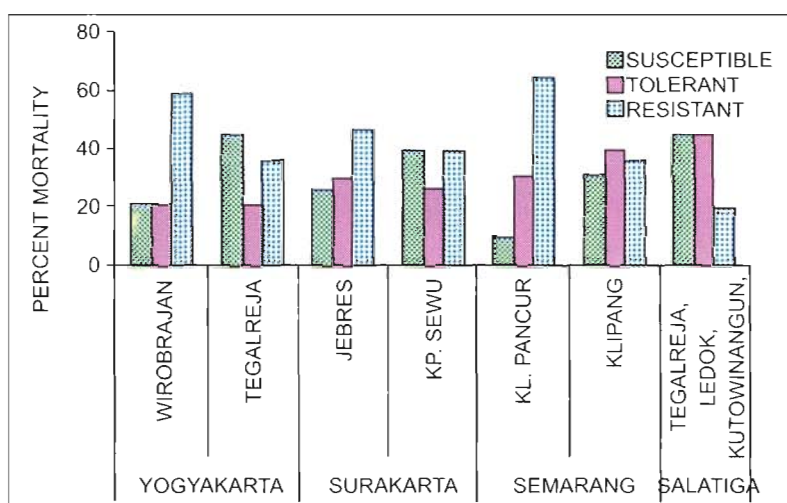


Figure 1. Results of biochemical/esterase (%) againts *Aedes aegypti* larvae which were collected from several areas of 'Joglosemar' and Salatiga.

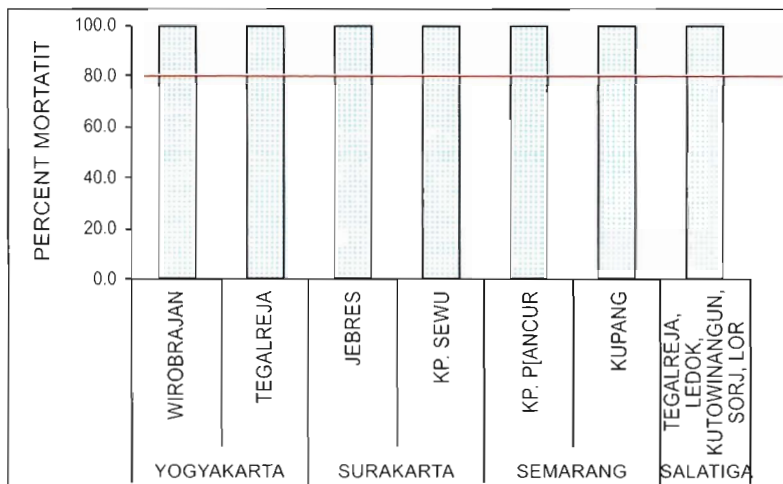


Figure 2. Results of susceptibility tests, percent (%) mortality of *Aedes aegypti* which were collected from several areas of ‘Joglosemar’ and Salatiga, against malathion 0.5 and 0.8%.

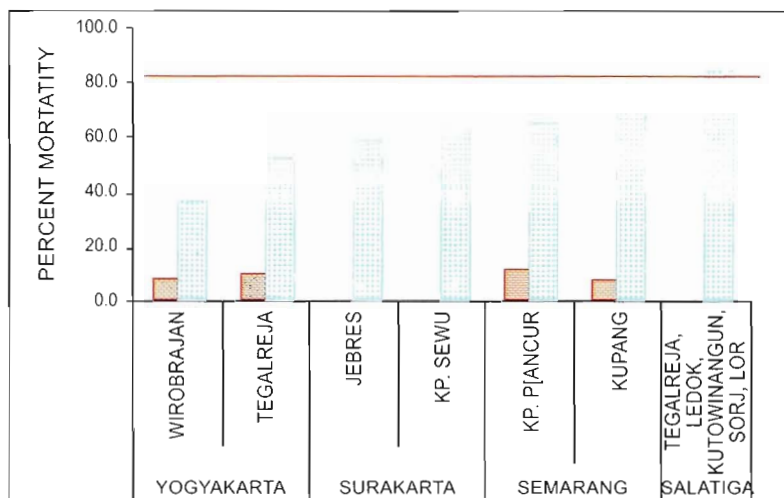


Figure 3. Results of bioassay tests, percent (%) mortality of *Aedes aegypti* larvae which were collected from several areas of ‘Joglosemar’ and Salatiga, against temephos 1% SG (dosage 1g/10 liters water).

The bioassay test of *Ae. aegypti* against Abate (Temephos 1% SG) showed that the population taken from Wirobrajan and Tegalrejo sub district were still susceptible against Temephos 1% SG (1 g/10

liters water) and caused 100.00% mortality (Figure 3).

Bioassay test/Esterase, samples collected from Salatiga, Central Java province

Samples which were collected from Tegalrejo, Ledok, Kutowinangun and Sidorejo

Lor villages (48 individual samples) showed that 41.70% *Aedes aegypti* larvae were still susceptible, 41.70% tolerant and the rest (16.60%) had been resistant against Organophosphate insecticides.

The results of susceptibility test of *Ae. aegypti* mosquitoes which was done into the samples collected from Tegalrejo, Ledok, Kutowinangun and Sidorejo Lor villages (using malathion impregnated paper) showed that on 0.5% concentration caused 0.00% mortality, while on 0.8% concentration had caused 83.00% mortality. These results considered that the population were tolerant against malathion (100 tested mosquitoes).

The bioassay test of *Ae. aegypti* against temephos 1% SG showed that the population taken from Tegalrejo, Ledok, Kutowinangun and Sidorejo Lor villages were still susceptible against temephos 1% SG (1 g/10 liters water) and caused 100.0% mortality (Attachment 1).

The results of bioassay and esterase tests concluded that (33.33%-62.50%) the larvae vector of *Ae. aegypti* on Joglosemar areas were resistant against Organophosphate insecticides (except Salatiga area; 16.60%).

The results of susceptibility test of *Ae. aegypti* mosquitoes which was done into the samples collected from Kalipancur sub district (using malathion and impregnated paper) appeared as: on 0.8% concentration made 36.0-74.0% mortality (in "Joglosemar" areas). Generally, the tested species from Salatiga were still tolerant (mortality: 83.00%) against malathion⁽²⁾. Research of the research showed that, the species population were still susceptible against Abate (temephos 1% SG) and had caused 99.00-100.00% mortality.

VRCRU Salatiga used *Ae. aegypti* (laboratory colony) as untreated control

(UTC). This study concluded that; 100% were still susceptible against Organophosphate insecticide (on the biochemical test) and 100.0% mortality after using 0.8% malathion impregnated paper (on bioassay test against temephos 1% SG; dosage 1 g/10 liters water) (Attachment 1).

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Attachment 1. Result of Results of Resistant Status of Dengue Haemorrhagic Fever (*Aedes Aegypti*) Study was Conducted by: Biochemical Analysis and Susceptibility Tests Method Samples were Collected From Several Areas of Central Java and Yogyakarta Provinces.

Study Areas	Number of tested mosquito larvae	BIOCHEMICAL TEST/ESTERASE (%)			Total tested mosquitoes	Susceptibility test mosq. Mortality (%)*		Bioassay against temefos 1% SG*	
		Susceptible	Tolerance	Resistant		Malathion (%) concentration		Number of tested larvae	Number of mortality (%)
						0.50	0.80		
Town : Yogyakarta									
Sub district Wirobrajan	48	20.83	20.83	58.33	100	8.0	36.0	100	99.0
Sub district Tegalrejo	48	43.75	20.83	35.41	100	10.0	52.0	100	100.0
Town : Surakarta									
Sub district Jebres	48	25.0	29.16	62.50	100	0.0	58.0	100	100.0
Sub district Kp. Sewu	48	37.5	25.0	37.50	100	0.0	63.0	100	100.0
Town : Semarang									
Sub district Kalipancur	48	8.34	29.16	62.50	100	12.0	65.0	100	100.0
Sub district Klipang		29.16	37.5	33.33	100	8.0	74.0	100	100.0
Town : Salatiga									
Village : Tegalrejo, Ledok, Kutawinangun dan Sidorejo Lor	48	41.70	41.70	16.60	100	0.00	83.00	100	100.0
Colony Lab. VR CRU Salatiga									
Lab. Code BR **	48	100.0	0.00	0.00	100		100.00	100	100.0
Lab. Code DM **	48	100.0	0.00	0.00	100		100.00	100	100.0

*) Mortality : 100%, the population is still susceptible ; 80% - 98% the population is tolerant ; < 80% the population is resistant (Herald, 1997)

**) Susceptible strain of *Ae. aegypti*