Berkala Ilmu Kedokteran
Vol. 27, No. 2, Juni 1995

Microplate assay analysis of potential for organophosphate insecticide resistance in *Aedes aegypti* in the Yogyakarta Municipality, Indonesia.

Sugeng Juwono Mardihusodo
Department of Parasitology, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia.

ABSTRAK

Sugeng Juwono Mardihusodo – *Analisis uji mikroplat terhadap potensi resistensi insektisida organofosfat pada Aedes aegypti di Kotamadya Yogyakarta, Indonesia.*

Sejak tahun 1970-an insektisida organofosfat (OP), temefos dan malathion, digunakan dalam program nasional pengendalian wabah demam berdarah Dengue di Indonesia, yang vektor utamanya adalah nyamuk Aedes aegypti. Dalam kurun waktu yang sama kedua senyawa OP itu diketahui menimbulkan resistensi pada Ae. aegypti stadium dewasa dan larva di Malaysia. Timbul dugaan bahwa Ae. aegypti stadium larva dan dewasa di Kodya Yogyakarta juga telah resisten terhadap kedua senyawa OP tersebut. Penelitian ini bertujuan untuk menetapkan status kerentanan Ae. aegypti stadium larva di Kodya Yogyakarta terhadap temefos dan malathion, dan mengetahui potensinya untuk menjadi resisten terhadap insektisida OP dibandingkan dengan populasi Ae. aegypti hasil kolonisasi di insektarium. Cara penelitian meliputi penggunaan uji mikroplat untuk peningkatan aktivitas enzim esterase non-spesifik pada Ae. aegypti stadium larva, diperkuat dengan uji hayati terhadap malathion dan temefos. Dari analisis data hasil penelitian tersebut disimpulkan, bahwa Ae. aegypti di Kodya Yogyakarta, stadium larvanya berkesan mulai resisten terhadap malathion dan temefos. Hal ini berbeda nyata dari Ae. aegypti stadium larva hasil kolonisasi insektarium yang masih sangat rentan terhadap kedua insektisida OP tersebut. Ae. aegypti di Kodya Yogyakarta berpotensi untuk menjadi resisten terhadap insektisida OP.

Key Words : biochemical test – malathion – temephos – insecticide resistance – *Aedes aegypti*

(Berkala Ilmu Kedokteran Vol. 27, No. 2, Juni 1995)

INTRODUCTION

Resistance of vectors to pesticides has continued to spread and affect disease control programmes in many countries. During the year of 1971-1980 there was 2.65 fold increase in the number of species of arthropods, from 313 to 829 species, developing resistance to all pesticide groups (DDT, cyclodiene, organo-phosphate, carbamate, pyrethroid, fumigant and others) as noted by Georghiou & Mellon.[1] Thereafter at least 89 species of mosquitoes (Diptera: Culicidae) were reported to be resistant to one or more compounds of all the commonly used groups of insecticides, among them was *Aedes aegypti*.[2] In 1984 Lee *et al.*[3] reported the occurrence of resistance in field-collected *Ae. aegypti* larvae in Kuala Lumpur, Malaysia, to Abate (temephos). Three years later, Lee *et al.*[4] stated that field-collected adults *Ae. aegypti* in the city were resistant to malathion. The two organophosphate (OP) insecticides have been widely used since 1973 in Malaysia.

Similar situation actually occurs in Yogyakarta and other towns in Java, where malathion and temephos have been commonly used for controlling *Ae. aegypti* to stop the transmission of Dengue Haemorrhagic Fever (DHF) since 1974.

The long term intensity of selection pressures on the target insect, adults and larvae of *Ae. aegypti* in Yogyakarta, by the two OP insecticides could be one of possible factor related to the development of resistance in the dengue vector. Such implications should be considered, and the potential for developing OP insecticide resistance in *Ae. aegypti* in Yogyakarta, and other densily populated urban areas in Java should be determined. However, such important issues are not fully realized, and often beyond the scope of the disease vector control programmes. Some hindering factors affecting such activities in the country might be: (a) lack of insectary facilities and shortage of technical personells for mosquito colonization; (b) relatively long period waiting to obtain sufficient number of mosquitoes for conducting the susceptibility tests, and (c) lack of insecticides and proper equipments for laboratory bioassays.[5] Therefore, it is obvious that development of methods of insecticide susceptibility tests, that are simple and rapid but sensitive and reliable, are highly necessary and would greatly facilitate monitoring activities in the field. Such ideal methods allow remedial actions to be implemented before the development of resistance in a particular target insect vector becomes a serious problem. At a critical time, i.e. the occurence of an epidemic of DHF in Yogyakarta, data on the susceptibility status of the target Dengue vector should be timely in hand prior to determining policy of consistency using malathion or temephos for chemical control, or shifting to the other methods of vector control as the alternative to the OP insecticides.

Microplate assay is one of several colorimetric biochemical methods to detect resistance in insects to OP insecticides due to the presence of elevated non-specific esterase activity therein, one of the mechanisms of developing resistance in the insects including mosquitoes.[5,6,7] Brogdon[8] states that biochemical microplate assays are cheaper and easier to use, and permit up to 30 assays to be made on a single insect and also give more reproducible results.

The present paper reports the results of series of laboratory studies aimed at determining: (a) the susceptibility status of *Ae. aegypti* larvae collected in the Yogyakarta Municipality to either malathion or temephos, and (b) the potential for OP resistance due to elevated non-esterase activities in the *Ae. aegypti* larval stages compared to that of insecticide resistance tests in *Ae. aegypti* larval stages of the laboratory stock colony as the controls, under laboratory conditions.

It is hoped that the following results would be useful to improve the control methods of Dengue vector, particularly in Yogyakarta, in the future.

MATERIALS AND METHODS

Materials

Mosquitoes. *Ae. aegypti* mosquitoes of indigenous strain of Yogyakarta were subjected for resistance tests. They were collected from different localities through ovitrap surveys. After hatching, the larvae were colonized in the laboratory until the adults emerged and the species differentiated or confirmed. Colonization of the mosquitoes were continued to obtain the F_1 generations of larval stages. The test larval stages were late third or early fourth instars. The same qualification were also applied to mosquitoes of laboratory strain colony that had been freed from insecticides in the laboratory, Faculty of Medicine, Gadjah Mada University, since 1986.[9] These were meant as the susceptible controls.

Insecticides. Organophosphorous insecticides used for the tests were malathion and temephos (Abate). They were applied at their respective diagnostic dosages to *Ae. aegypti*, larvae namely 0.02 mg/l and 1.0 mg/l for malathion and temephos respectively, as recommended by the WHO.[2]

Chemicals. Main chemicals used for enzymatic assays were (a) substrate solution: 0.5 ml α-naphthyl [acetate in aceton (6 g/l)] mixed with 50 ml phosphate buffer solution (0.02 M; pH=7.0), and (b) coupling reagent: 150 mg of Fast Blue B. salt in 15 ml water and 35 ml aqueous (5%; W/V) sodium dlodecyl sulphate.

Equipments. Main equipments for bioassays of mosquito larvae were paper cups of 250 ml. A glass rod, a micropipette of 50 µ, microplates with flat bottomed wells, and an ELISA Reader Titertek Multiskan (MCC/340) were the main equipments used for the biochemical tests (microplate assays).

Methods

Microplate assay. Homogenates from larval stages of *Ae. aegypti* mosquitoes were screened for α-naphthyl acetate hydrolyzing esterase (α-NA Est) activity using a method described by Lee [5]. Whole body of individual larva was used for all experiments. Single larva was first homogenized in 0.5 µl PBS using a glass rod. With a micropipette, 50 of the clear homogenate was transferred to a well in a microtitre plate. Using this procedure 8 replicate alliquots of the homogenate from a single specimen were available for assay. Fifty µl of the substrate solution freshly prepared were then pipetted into each well and left for 60 seconds. The coupling reagent (50 µl) was then added. Immediately a deep purple color developed which turned to blue after standing for 10 minutes. The reaction was stopped by adding up of 50 µl 10% acetic acid into each well. The intensity of the final color, indicative of esterase activity, could be differentiated by eye and was assigned the following scores : 0 = colorless/very faint blue; 1=faint/light blue; 2=greenish blue; 3=dark blue. The intensity of the final color was also scanned by an ELISA reader at γ=450 nm to determine the color intensity quantitatively.

Bioassay. The susceptibility status of *Ae. aegypti* larvae to malathion and temephos was determined by exposing the larvae to diagnostic dosages of the two OP insecticides which were 1.0 mg/l and 0.02 mg/l respectively.[2,10] In brief, an appropriate volume of an insecticide solution was introduced into paper cups and 25 larvae were added. The solution was then topped up to 250 ml with distilled water. A small amount of larval food were added, and larval mortality was recorded after 24 hours. Three replicates were used for each series of resistence test, and whenever possible each test concentration was repeated at least 3 times on the same day, in a room of more or less same conditions room (t=26° ± 2°C; RH=68 ± 4%), for all experiments.

Data interpretation and analysis.

Microplate assay data of α- NA esterase in *Ae. aegypti* larvae showing the resistance status to OP insecticides were interpreted in accordance with experimental evidence for eye score of final color intensity of the enzymetric reactions obtained by Lee [5] as follows : 0 to 2.0 was highly susceptible (SS); 2.0 to 2.5 was moderately resistant (RS), and 2.6 to 3.0 was highly resistant (RR).

Bioassay data of *Ae. aegypti* larvae to malathion and temephos of its respective diagnostic dosages, that were mortality rates of the test larvae after 24 hr exposure time, were interpreted according to the criteria of susceptibility/ resistancy status of the test mosquitoes proposed by Davidson & Zahar[11] as follows: mortality rate (%) ≥ 98% = susceptible (SS); 80-98% = tolerant or moderate resistant (RS), and ≤ 80% = resistant (RR). Potential for OP insecticide resistance in *Ae. aegypti* was shown by the presence of moderate resistance (RS) and resistance (RR) as indicated by significantly lower number (%) of the test mosquito larvae of each locality under study exposed to malathion and temephos of the diagnostic dosages compared to the control (laboratory strain) which were judged with χ^2-test at α=0.05.

RESULTS

Microplate assays

A total of 106 larvae of *Ae. aegypti* of wild field strain (Yogyakarta) together with 22 mosquito larvae of laboratory strain were assayed for qualitative determination of α-NA Est activities that were considered to be related to organophosphate insecticides (TABLE 1). As expected, all samples of mosquito larvae of the laboratory strain (176 replicates of 22 larvae) were susceptible to OP insecticides (OP) based on the average color score (1.98) less than 2.0. Such score indicated no or very low activity of the non-specific Est to hydrolyze toxicant, i.e. some OP insecticides, and permits the major portion of the active ingredient of the toxicant through a specific mechanism to kill the target insect.

Microplate assays of the larval samples from fields showed slight different results. Thirty four mosquito larvae sampled from Mergangsan (272 replicates of the homogenates) showed average color score of 1.98 or less than 2.0 meaning that the mosquito larvae were highly susceptible (SS), while the others: 24 (192 replicates of the

TABLE 1. – Qualitative determination of α-napthyl acetate hydrolysing esterase (α-NA Est) activities by microplate assays in *Aedes aegypti* larvae collected from different localities in Yogyakarta Municipality.

Population Locality (District)	Esterase reaction and susceptibility status			
	Total larvae	Total replicate*	Average color score**	Susceptibility status***
Control:				
Laboratory	22	176	1.25	SS
Field (Yogyakarta):				
Mergangsan	34	272	1.98	SS
Ngampilan	24	192	2.11	RS
Tegalrejo	24	192	2.33	RS
Umbulharjo	24	192	2.24	RS
All fields	106	848	2.17	RS

* Each replicate was 50 ml homogenate from a single larva

** Eye Score : 0 = colorless/very faint blue
1 = faint/light blue
2 = greenish blue
3 = dark blue

*** Susceptibility status criteria by Lee[5] for eye color score:
2.0 = no α-NA Est activity=highly susceptible (SS)
2.0 -2.5 = low α-NA Est activity=moderately resistant (RS)
2.6 -3.0 = high α-NA Est activity=highly resistant (RR)

homogenates) mosquito larvae, from Ngampilan, Tegalrejo and Umbulharjo, showed average color score of 2.11, 2.33 and 2.24 respectively or between 2.0-2.5, meaning that the mosquito larvae from the last three localities were tolerant or moderately resistant (RS). Potentials resistance to OP insecticides in *Ae. aegypti* mosquito larvae were likely present in Ngampilan, Tegalrejo and Umbulharjo, and less likely in Mergangsan. Thus, in majority of cases from all field localities 106 mosquito larvae (848 replicates of the homogenates) in average showed color score of 2.17 or between 2.0 to 2.5, meaning that in average *Ae. aegypti* larvae from the localities (Yogyakarta) showed potentials for OP resistance due to the presence of elevated esterase (α-NA Est) activities.

Esterase activities in hydrolysing a substrate, i.e. α-NA, could be determined quantitatively by measuring the color intensity with an ELISA reader at a certain light microwave length, i.e. 450 nm, as at the present studies. Results of such works on the microplate enzymatic reactions are presented in TABLE 2 and FIGURE 1. These presentations add further verifications on the potentials for OP resistance related to the elevation of α-NA Est activities in *Ae. aegypti* larvae collected in Yogyakarta Municipality, that was more evidence in Umbulharjo than in the other three localities (Mergangsan, Ngampilan and Tegalrejo). The levels of the Est activities of all larvae of the laboratory strain were read at the absorbance value (AV) range between 0.201 to 0.400, while all mosquito larvae collected from the all fields were at the AV range of 0.301 to 0.1200.

From the present studies it is known that any α-NA Est reactions in the wells of microplates showing colorless/very faint blue to faint/light blue and read AVs 0.700, and that showing greenish blue to normal blue and read at AVs of 0.701 to 0.900, and that showing dark blue color and read at AVs ≥ 0.901, correspond to eye color score of < 2.0 for susceptible (SS), of 2.0 to 2.5 for moderately resistant (RS), and of 2.6 to 3.0 for highly resistant (RR) respectively. Such empirical correspondence between the AVs ranges and eye color scores classifying the susceptibility status of the test insects under studies are presented in TABLE 3. Potentials resistance for OP insecticide due to elevated Est activities were found among mosquitoes from the fields but that from Umbulharjo were the most prominent, namely 91.67% of total replicates (192) compared to 1.48%, 54.12% and 39.17% of total replicates from Mergangsan, Ngampilan and Tegalrejo respectively, as tested with χ^2-test.

Bioassays

Results of bioassays to determine the susceptibility status of *Ae. aegypti* of larval stages collected from Yogyakarta using malathion and temephos of the diagnostic dosages are presented

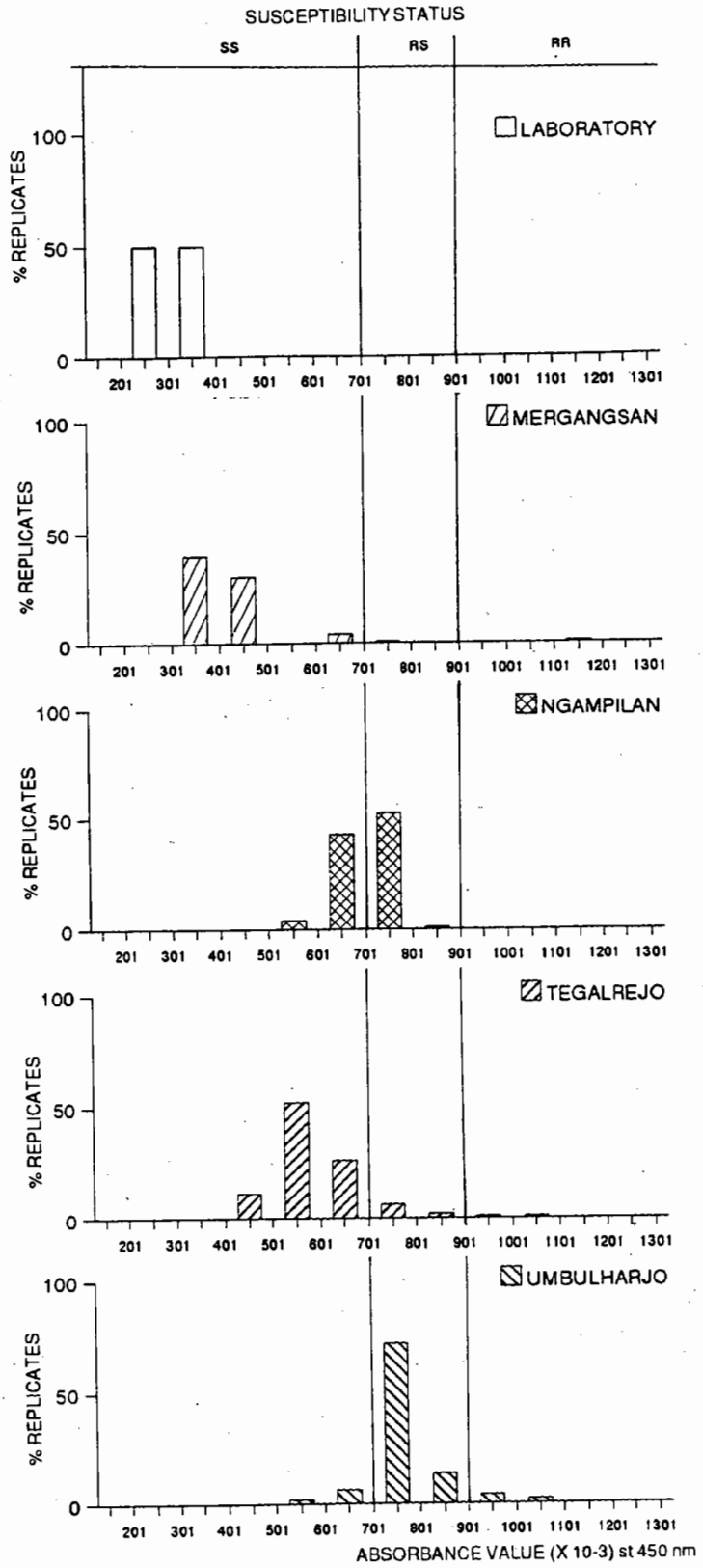

FIGURE 1. – Absorbance values (AVs) of colour intensities showing α-naphthyl acetate lydrolysing esterase activities in different replicates of homogerates from *Aedes aegypti* larvae of different population in the Yogyakarta Municipality, Indonesia.

TABLE 2. – Quantitative determination of α-naphthyl acetate hydrolysing esterase activities in *Aedes aegypti* larvae from Yogyakarta by using microplate assays and measured with ELISA reader at γ=450 nm.

Range of AV* ($\times 10^{-3}$)	Laboratory	Mergangsan	Ngampilan	Tegalrejo	Umbulharjo
	No. Replic. (%)	No. Replic. (%)	No. Replic. (%)	No. Replic. (%)	No. Replic. (%)
210 – 300	88 (50)	–	–	–	–
301 – 400	88 (50)	108 (39.71)	–	–	–
401 – 500	–	82 (30.14)	–	22 (11.44)	–
501 – 600	–	–	8 (4.17)	100 (52.09)	4 (2.08)
601 – 700	–	78 (4.17)	82 (42.71)	50 (26.04)	12 (6.25)
701 – 800	–	2 (0.74)	100 (52.08)	12 (6.25)	138 (71.88)
801 – 900	–	–	2 (1.04)	4 (2.08)	26 (13.55)
901 – 1000	–	–	–	2 (1.04)	8 (4.16)
1001 – 1100	–	–	–	2 (1.04)	4 (2.08)
1101 – 1200	–	2 (0.74)	–	–	–
≥ 1201	–	–	–	–	–
201 – 1201	176 (100)	272 (100)	192 (100)	192 (100)	192 (100)
No. of larvae	22	34	24	24	244

* AV = absorbance value

TABLE. 3. – Quantitative determination of α-naphtyl acetate hydrolysing esterase activities by microplate assays related to susceptiblity status of *Aedes aegypti* from Yogyakarta to organophosphorous insecticides measured with ELISA reader at γ=450 nm

Population Locality (District)	Total larvae (Replicates)	Frequency (%) and Susceptibility Status* in Esterase Reaction		
		AV** ≤ 0.700	AV=0.701-0.900	AV≥ 0.901
		SS	RS	RR
Control :				
Laboratory	22 (176)	100	0	0
Fields				
Mergangsan	34 (272)	98.52	0.74	0.74
Ngampilan	24 (192)	46.88	54.12	0
Tegalrejo	24 (192)	89.57	8.35	1.04
Umbulharjo	24 (192)	8.33	88.55	3.12
All fields	106	60.83	37.94	1.23

* Susceptibility status, based on the empirical significances obtained from the present studies i.e:
(a) AV<0.700 is equal to average color score<2.0, meaning highly susceptible (SS)
(b) AV=0.700-0.900 is equal to average color score 2.0-2.5, meaning moderate resistance (RS)
(c) AV>0.900 is equal to average color score 2.6-3.00, meaning highly resistant (RR)

** AV = absorbance value

in TABLE 4. The laboratory strain of *Ae. aegypti* mosquito larvae were susceptible to both malathion and temephos, while the wild strain collected from different localities in the Yogyakarta Municipality showed some degree of resistance to the OP insecticides. Exposed to 1.0 mg/l malathion for 24 hours under laboratory conditions (T=26° ± 2°C; RH=78% ± 4%) larval mortality of *Ae. aegypti* from Mergangsan and Ngampilan were 98.6% and 85.3% respectively, meaning that the mosquito larvae were susceptible (SS) and moderately resistant (RS) respectively, while that from Tegalrejo and Umbulharjo were 72.6% and 75.3% respectively. These findings showed that the mosquito larvae in the two localities were highly resistant (RR) to the OP insecticide. In average, as the mosquitoes from the four localities were very likely sympathric species, the larval mortality was 82.95% meaning that they were moderately resistant (RS) to malathion.

Exposed to 0.02 mg/l temephos under the same laboratory conditions of bioassays, mortality of *Ae. aegypti* larvae from Mergangsan and Ngampilan were 92.3% and 71.6% respectively, meaning that the mosquito larvae in the two

TABLE 4. – Bioassays of *Aedes aegypti* mosquitoes of larval stages from Yogyakarta using diagnostic dosages of malathion and temephos under laboratory conditions (T=26° ± 2°C; RH=78% ± 4%)

Population Locality (District)	Larval Mortality (%) and Susceptibility[1)]			
	Malathion	(1.0 mg/l)	Temephos	(0.02 mg/l)
Control:				
Laboratory	100	SS[2)]	100	SS
Fields (Yogyakarta):				
Mergangsan	98.6	SS	92.3	RS[3)]
Ngampilan	85.3	RS	71.6	RR[4)]
Tegalrejo	72.6	RR	89.3	RS
Umbulharjo	75.3	RR	85.6	RS
All fields	82.95	RS	84.7	RS

[1)] Determined according to Davidson and Zahar[11], [2)] SS = highly susceptible, [3)] RS = moderately resistant, [4)] R = highly resistant

localities were moderately resistant (RS) and highly resistant (RR) respectively, while that from Tegalrejo and Umbulharjo were 89.3% and 85.6% respectively, meaning that the mosquito larvae from the two localities were moderately resistant (RS) to the OP insecticide. In average, as the mosquitoes from the four localities were also sympatric, the larval mortality was 84.7%, meaning that in general they were moderately resistant (RS) to temephos.

In conclusion, bioassays of *Ae. aegypti* larvae from the four localities in Yogyakarta Municipality (Mergangsan, Ngampilan, Tegalrejo and Umbulharjo) were moderately resistant (RS) to both malathion, and temephos.

DISCUSSION

Qualitative determination of α-NA Est activities by microplate assays in *Ae. aegypti* larvae collected from different districts in the Yogyakarta Municipality, indicate moderated level of insecticide resistance in the mosquito larvae (TABLE 1). This implied that the field population of *Ae. aegypti* under the study comprised at least two subpopulations, i.e susceptible and resistant. Further quantitative determination of the Est activities by measuring the absorbance values (AVs) of the color intensities of the enzymatic reaction in microplate wells with ELISA reader at =450 nm revealed variation in the range of the AVs, i.e. mosquito larvae from Mergangsan and Umbulharjo were 0.301 to 0.1200, and 0.501 to 0.1100 respectively (TABLE 2). Such evidence showed the heterogenity of *Ae. aegypti* population in the fields, and reflected the presence of genotypic polymorphism in the population of mosquitoes particularly in relation to the detoxification of esterase genes.

Frequency (%) of susceptibility level (SS, RS and RR) were estimated from comparative eye score/absorbance value (=450 nm) data on *Ae. aegypti* under study, i.e: (a) AV corresponds to average color score, meaning susceptible (SS); (b) AV=0.700-0.900 corresponds to average color score of 2.0-2.5, meaning moderately resistant (RS), and (c) AV=0.901 corresponds to average color score of 2.6-3.00, meaning highly resistant (RR) (TABLE 3). Such finding enabled more rapid determination of the susceptibility status of *Ae. aegypti* (larvae) to OP insecticides in case of metabolic resistance due to increased esterase activity. Brogdon *et al.* [7] made estimation of the percentage of insecticide resistance due to elevated non-specific Est from comparative bioassay/microassay data on *Anopheles albimanus* at absorbance 550 nm, and found that the resistance threshold in microplate assays of the mosquito was also 0.900. Lee[5] in his work on the detection of insecticide resistance due to elevated esterase activity in *Culex quinquefasciatus*, which were scanned with ELISA reader at =450 nm, concluded that the resistance threshold of the target insect vector under control programme is quite important.

Bioassay results of *Ae. aegypti* larvae from Yogyakarta using diagnostic dosages of mala-

thion and temephos under laboratory conditions (TABLE 4) reflected different responses of the mosquito larvae to the two insecticides. Such evidence as obtained by the microassays showed some heterogenities of *Ae. aegypti* in Yogyakarta, and were reflective of the presence of genotypic polymorphism in the mosquito population possibly underlying mechanism of resistance to the insecticides.

Overview on the results of microassays and bioassays of *Ae. aegypti* larvae from the fields (Yogyakarta) both to malathion and temephos revealed the presence of either consistency or inconsistency, i.e. consistency of the microassay and bioassay data on the susceptibility status of *Ae. aegypti* larva from Mergangsan to malathion, but inconsistensy of that to temephos (TABLE 1 and TABLE 4). These might be due to the same mechanism operationally underlying the resistance associated with enzyme activity, or different mechanisms such as insensitivity of acetyl-cholinesterase (AChE) or enhanced metabolism by hydrolase and multifunction oxydase.[12]

Intensitivity of AChE is the most common mechanism of insecticide resistance among the three plausible mechanisms other than increased esterase activity as reported by Brogdon *et al.*[7] and Hemingway & Smith.[13] Resistance due to the intensity of AChE in mosquitoes, i.e. *Ae. aegypti* could be due to intensive pressure of the insects with carbamates.[13]

Data on microassays and bioassays of *Ae. aegypti* larvae colonized in our laboratory showed that the laboratory strain were highly susceptible to the OP insecticides, malathion and temephos (TABLE 1-4). This is expected fulfilling the control group criteria for the present study. On the other hand, *Ae. aegypti* larvae collected from different localities (districts) in the Yogyakarta Municipality were in their susceptibility status to malathion and temephos. In average, *Ae. aegypti* larvae from the four districts were moderately resistant to both malathion and temephos. This conclusion coincides with the hypothesis that the long term intensity of selection pressure on the target insect, *Ae. aegypti* in Yogyakarta, directly by adulticide malathion and larvicide temephos in Yogyakarta, could be one of possible factors related to the development of resistance in the dengue vector. Herath & Davidson[14] from their study on malathion resistance in *An. culifacies* revealed at least two mechanisms of the insecticide resistance, one involving the specific carboxylesterase, and the other the less specific multifunction oxydase system. Hemingway[15] reported that malathion resistance in adult stages of *An. arabiensis* was synergized by triphenyl phosphate, but not by piperonyl butoxides, and suggested that a carboxylesterase enzyme might be the basis of malathion resistance. Lee[6] confirmed in his work on microassay of *Ae. aegypti* from Kuala Lumpur, Malaysia, that intensity of color of the esterase reaction, which reflected esterase level, correlated positively with eye score, which reflected the level of OP (temephos) resistance in *Ae. aegypti* larvae. He claimed that microplate assays for detection and monitoring of insecticide resistance particularly due to enhanced metabolism were quite sensitive, reliable, valid, and cheaper compared to bioassays even by using diagnostic dosages. Such statements coincide absolutely with that of Brogdon.[16] Recently Devonshire *et al.*[17] in their study on the biochemical resistance of aphids (*Myzus persicae*) using microplate assay stated that although microplate assay was a poorer discriminator compared to immunoassay (that discriminate resistant variants R_1 and R_2), but the biochemical method could identify most of the very resistant (R_2) aphids, and provided a robust and widely accessible method for representing the insecticide resistance of field insect population.

It seems to be no doubt that the microassay as used in the present study could detect, at least in part, could also be applied in other regencies in Indonesia, even in other insects/arthropods of either medical or agricultural importance. The ability to identify the occurrence of different resistance genotypes in field population of mosquito (including *Ae. aegypti* in Indonesia, where Dengue/DHF is endemic and vector control is implemented) is considered important for the purpose of optimising chemical control operations.[18] Rapid microassays of enzymes responsible for resistance provides a means for rapidly assessing genetic background of target mosquito population, like *Ae. aegypti*, in Yogyakarta Municipality.

CONCLUSION

Based on the microassay data of elevated α-NA Est activities in *Ae. aegypti* larvae from Mergangsan, Ngampilan, Tegalrejo and Umbulharjo districts in Yogyakarta Municipality, and bioassay of the mosquito larvae to malathion and temephos, it was concluded that *Ae. aegypti* larvae from the fields (Yogyakarta) were moderately resistant to both organophosphate insecticides, and showed potentials for developing resistance to the OP insecticides due to elevated α-NA Est activity.

ACKNOWLEDGEMENT

The author whises to thank the Rector of Gadjah Mada University for the financial support through Dana Penunjang Pendidikan Universitas Gadjah Mada (DPP UGM) under the Research Contract No. UGM/5950/M/09/01, October 1, 1993, Gadjah Mada University Research Institute.

REFERENCES

1. Georghiou GP, Roni RB. Pesticide resistance in time and space. In: Georghiou GP, Saito T, Editors.: Pest resistance to pesticides. New York: Plenum Press, 1983: 1-46.
2. WHO Expert Committee on Vector Biology and Control. Resistance of vectors and reservoirs of disease to pesticides. Geneva: WHO; 1986. WHO Tech Rep Ser No. 737.
3. Lee HL, Lee TW, Law FM & Cheong WH. Preliminary studies on the susceptibility of field-collected Aedes (stegomyia) aegypti (Linneaus) to Abate (Temephos) in Kuala Lumpur. Trop Biomed 1984; 1: 37-40.
4. Lee HL, Kasemsri T, Cheong WH. Laboratory evaluation of resistance status of field-collected Aedes (Stegomyia) aegypti (Linneaus) to malathion in Kuala Lumpur. Trop Biomed 1987; 4: 192-95.
5. Lee HL. A rapid and simple biochemical method for detection of insecticide resistance due to elevated esterase activity in Culex quinquefasciatus. Trop Biomed 1990; 7: 21-8.
6. Lee HL. Esterase activity and temephos susceptibility in Aedes aegypti (L.) larvae. Mosquito-Borne Dis Bull 1991; 8: 91-94.
7. Brogdon WG, Beach RF, Stewart JM, Castanaza L. Microplate assay analysis of the distribution of organophosphate and carbamate resistance in Guatemalan Anopheles albimanus. Bull WHO 1988; 66: 339-46.
8. Brogdon WG. Biochemical resistance detection: An alternative to bioassay. Parasitology Today 1989; 5: 56-60.
9. Mardihusodo SJ, Untung K, & Sularto T. Uji lapangan skala kecil tentang pengaruh kabut panas chlorpyrifos terhadap nyamuk Aedes aegypti dan Anopheles aconitus di Yogyakarta. B I Ked 1988; 20: 9-19.
10. WHO Expert Committee on Insecticides. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. Geneva: WHO; 1981 WHO/VBC/81.807.
11. Davidson G, Zahar AR. The practical implications of resistance of malaria vectors to insecticides. Bull WHO 1973; 49: 475-83.
12. WHO Expert Committee on Vector Biology and Control. Resistance of vectors of disease to pesticides. Geneva: WHO; 1080. WHO Tech Rep Ser 655.
13. Hemingway J & Smith C. Field and laboratory detection of the altered acetylcholinesterase resistance genes which confer organophosphate and carbamate resistance in mosquito (Diptera: Culicidae). Bull Ent Res 1986; 76:559-65.
14. Herath PRJ, Davidson G. The nature of malathion resistance in a population of Anopheles culifacies Giles. Bull WHO 1981; 59: 383-86.
15. Hemingway J. Biochemical studies on malathion resistance in Anopheles arabiensis from Sudan. Trans Roy Soc Trop Med Hyg 1983; 77: 477-80.
16. Brogdon WG. Biochemical resistance detection : An alternative to bioassay. Parasitology Today 1989; 5: 56-60.
17. Devonshire AL, Devine GJ, Moores GD. Comparison of microplate esterase assays, and immunoassay for identifying insecticide resistant variants of Myzus persicae (Homoptera: Aphididae). Bull Ent Res 1992; 82: 459-63.
18. Lee HL & Todano T. Monitoring resistance gene frequencies in Malaysian Culex quinquefasciatus Say adults using rapid non-specific esterase enzyme microassays. South East Asian J Trop Med & Pub Hlth 1994; 25: 371-73.