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Abstrak

Latar Belakang: *Resistensi terhadap insektisida terutama terjadi karena adanya perubahan pada enzim metabolik serangga. Enzim metabolik yang sering berperan dalam kejadian resistensi antara lain adalah esterase dan monooksigenase.*

Metode: *Uji kerentanan dan uji biokimia untuk mendeteksi resistensi terhadap malation dan cypermetrin dilakukan pada Aedes aegypti dari Wonosobo (daerah endemis baru infeksi Dengue di dataran tinggi). Uji coba yang dilakukan pada generasi F1 nyamuk Ae.aegypti yang tertangkap di lapangan bertujuan untuk mengetahui mekanisme resistensi berdasarkan aktivitas dua enzim detoksifikasi yaitu esterase dan monooksigenase. Wawancara dengan menggunakan kuesioner terstruktur dilakukan untuk mengetahui penggunaan insektisida oleh masyarakat dan pemerintah daerah.*

Hasil: *Uji kerentanan menunjukkan mortalitas sebesar 23,4% setelah terpapar malathion 0,8% dan 46,7% setelah terpapar cypermethrin 0,05%. Hasil uji biokimia menunjukkan bahwa aktivitas esterase dan monooksigenase cenderung meningkat pada Ae.aegypti di Wonosobo. Wawancara dan kuesioner menyimpulkan bahwa sintetik piretroid adalah satu-satunya golongan insektisida yang digunakan dalam program pengendalian vektor oleh Dinas Kesehatan Wonosobo dan merupakan tipe insektisida yang paling sering digunakan di rumah tangga oleh masyarakat Wonosobo untuk mengendalikan populasi Ae.aegypti.*

Kesimpulan: *Ditemukan nyamuk Ae. aegypti yang mengalami peningkatan aktivitas enzim esterase dan monooksigenase pada populasi Ae. aegypti di Kabupaten Wonosobo. Hal ini selaras dengan status resistensi populasi nyamuk tersebut yang resisten terhadap Malation dan Cypermetrin.* ***(Health Science Journal of Indonesia 2017;8(2):74-80)***

Kata kunci: *Ae.aegypti Wonosobo, biokimia, paparan insektisida, resistensi*

Abstract

Background: Resistance to insecticides mainly occurs due to changes in insect metabolic enzyme. Metabolic enzyme which were often involved in insecticide resistance are esterase and monooxygenase.

Methods: Susceptibility test and biochemical assay to detect malathion and cypermethrin resistance were conducted on *Aedes aegypti* from Wonosobo (new highland Dengue endemic area). The test were performed on F1 generation of *Ae.aegypti* field caught mosquitoes which aimed to determine the resistance mechanisms regarding two detoxifying enzymes i.e. esterase and monooxygenase. Interview using structured questionaires was conduted to investigate the usage of insecticide by the society and local government.

Results: Susceptibility test showed 23.4 and 46.7% mortalities after exposure to 0.8% malathion and 0.05% cypermethrin. The biochemical assay result suggested that esterase, and monooxygenase activity tend to increase in *Ae.aegypti* in Wonosobo. Interview and questionaires conclude that synthethic pyrethroid was the only insecticide type used in vector control program by Wonosobo Health Office and was the most frequent insecticide type to be used in household by Wonosobo society to control *Ae.aegypti* population.

Conclusion: *Aedes aegypti* with increased esterase and monooxygenase activity were found in Wonosobo. This result was in line with the resistance status of *Ae. aegypti* population in Wonosobo which resistant to Malathion and Cypermethrin. ***(Health Science Journal of Indonesia 2017;8(2):74-80)***

Keywords: *Ae.aegypti* Wonosobo, biochemical, insecticide exposure, resistance

Dengue haemorrhagic fever is still one of the major mosquito borne diseases in Indonesia with *Aedes aegypti* as the principal vector. *Aedes aegypti* is closely associated with humans and human habitation. The female is predominantly an indoor day-biter that feeds almost exclusively on humans. Adult female usually exploits artificial containers as sites to deposit her eggs. *Aedes aegypti* mosquitoes are abundant and endemic dengue virus transmission usually occurs in low-elevation areas.[1] But recently a large proportion of the human population lives in high-altitude cities. On the other side, due to the rising global temperature, more areas of the world become favourable for the survival of tropical insects like *Ae. aegypti*. This could lead to the spread of this insect towards more high elevation areas thus supporting the expansion of dengue endemic areas.[2] Wonosobo is one of dengue endemic district in Central Java Province, is a mountainous area with altitude ranges from 250 m to 2,250 m above sea level. Dengue fever was reported in Wonosobo since 2007. [3] Vector control by reducing *Ae.aegypti* population is at present the only viable option available to control the disease.

Especially during epidemics of the disease, the use of insecticides is needed. For example, application of temephos for larval control, thermal fogging or ULV sprays of certain organophosphates such as malathion and synthethic pyrethroids such as cypermethrin for adult control. Fogging with cypermethrin was always done in the last five years by the government as an effort to reduce Dengue vectors in Wonosobo.[4] Insecticide resistance could develop and would be a major problem in controlling the vectors and other pest insects.

The major metabolic enzymes involved in insects resistance include P450 mediated monooxygenases, elevated non-specific esterases, and glutathione s-transferase.[5,6,7,8,9,10] Moreover, increased levels of monooxygenase have been associated in *Ae.aegypti* resistance.[11] Some previous studies has documented about elevated detoxification enzyme activities in *Ae. aegypti* population in Central Java.[12,13] In this study, we conducted susceptibility assay using malathion 0,8% and cypermethrin 0,05% for detection of resistance in *Ae.aegypti* from Wonosobo, a new Dengue endemic area in highland. The assay was integrated with biochemical assays of enzymes to define the underlined mechanisms involved in malathion and synthethic pyrethroid resistance. Besides, interview with Public Health Officer and questionnaire survey among society were also conducted to obtain information about insecticide exposure to *Ae.aegypti* population in Wonosobo.

METHODS

Study area

Wonosobo regency is a mountainous area with an altitude between 250 m to 2,250 m above sea level, including the type of young mountains with steep valley. Dengue cases were found in this regency since 2007 and distributed in some municipalities (Figure 1). The altitude of these municipalities ranges from 300 to 900 m above sea level. Daily temperature ranges from 24°C – 30°C. The study was conducted in January-October 2014, included three Dengue endemic municipalities located in Wonosobo Regency i.e. Wonosobo, Selomerto and Leksono.

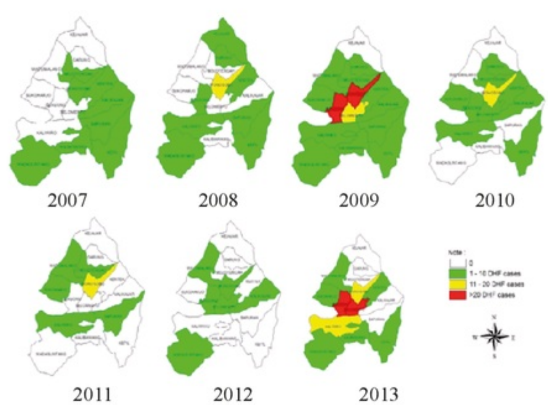

Figure 1. Distribution of DHF cases in Wonosobo District in 2007-2013

Figure 1 showed that DHF cases frequently found in Wonosobo, Leksono and Selomerto. In 2013, significant increasing cases occurred in Leksono and Selomerto.

Mosquito Samples

Stock of *Ae.aegypti* originated from field-collected egg from three Dengue endemic municipalities located in Wonosobo Regency i.e. Wonosobo, Selomerto and Leksono. These eggs were maintained in the cloth cages in an insectary at 28 °C and 80% RH with 12:12 day:night. Females were induced to lay eggs and subsequent colonies were reared through to adults. F1 progeny were divided into subsamples according to the number emerged. One subsamples was stored in deep freezer (-80 °C) to be used for biochemical analysis while the other subsamples were used for susceptibility assay.

Insecticide Exposure

The usage of household insecticide can trigger the occurrence of resistance because active ingredients in household insecticides are mostly derived from the synthetic pyrethroid. A questionnaire survey of 100 households was conducted to obtain information on usage of household insecticide in each village in Wonosobo. Interview with Public Health Officer was also conducted to collect information about the usage of insecticide in vector control activities by Regency Health Office.

Susceptibility assay

Adult insecticide susceptibility bioassays were carried out using WHO test kits for Malathion and Cypermethrin.[14] The insecticides impregnated and control (risella oil) papers were obtained from Vector Control Research Unit (VCRU), University Sains Malaysia, Malaysia.

Twenty five non-blood fed adult female *Ae.aegypti* aged 2-3 days old of Wonosobo strains were exposed to the diagnostic concentrations of cypermethrin 0.05% and malathion 0.8% impregnated paper respectively for 1 hour.[14] The mortality was recorded at the end of 24 hours holding period. Four replicates were conducted with controls exposed only to risella oil.

Biochemical test

Ninety mosquitoes were homogenized individually in 200 µl distilled water. Each of 20 µl of the homogenate was used for esterase and monooxygenase assay. The protocol for each assay followed WHO.[15] The blank plates contain 20 µl aquadest were used as negative control. The details of each assay were as followed;

Esterase assay. Twenty µl of homogenated were placed in separated wells of microtitre plate and 30 mM α-naphthyl acetate that had been previously prepared at a fixed quantity was added in each well. The plate was left at a temperature of 25±2 °C for 15 minutes. Following that, 0.1% fast blue-SDS was added and it was left at room temperature at 25±2 °C. The test was then carried out using a microplate reader and the absorbance value was read at 450 nm. The microplate assay for non-specific esterase hydrolizing α-napthyl acetate substrate were interpreted in correspondence with the experimental evidence for absorbance value of the final color intensity of the enzymatic reactions obtained by Mardihusodo, that was as follows: (1) esterase reactions which were colorless/faint blue were read at AV < 0.700; (2) esterase reactions which were greenish blue were read at 0.700-0.900, and (3) esterase reactions showing deep blue in color were read at AV > 0.900.[16]

Monooxygenase assay. A hundred microlitres of sodium phosphate buffer pH 7.2 was added to the aliquots of mosquito homogenates and 200 µg of 3,3,5',5'-tetramethhyl benzidine (TMBZ) solution (0.01 g of 3,3,5',5'-tetramethhyl benzidine in 5 ml of absolute methanol, mixed with 0.25M sodium acetate buffer pH 5.0) was added. Twenty-five microlitres of 3% hydrogen peroxide was added and the mixture was left for two hours at room temperature. The oxidase enzyme activity was then read at 630 nm. The microplate assay for monooxygenase were interpreted in correspondence with the experimental evidence for absorbance value of the final color intensity of the enzymatic reactions obtained by Matowo and, that was as follows: (1) monooxygenase reactions which were colorless/faint blue were read at AV < 0.165 and esterase reactions which were deep blue were read at AV > 0.165.[17]

Data analysis

Number of mosquito mortality in susceptibility test, absorbance value data from each microtitre plate and the usage of household insecticide were shown in percentage.

RESULTS

Susceptibility test

The adults of control group showed zero mortality to risella paper. While adults treated group were exposed to 0.8% malathion , 0.05% cypermethrin, the mortalities of 23.3 % and 46.7% were observed (Table 1).

Table 1. Susceptibility Test Result using 0.8% Malathion and 0.05% Cypermethrin

Insecticide	Life Stage	N (number of examined mosquito)	% mortality
Malathion 0.8% impregnated paper	Adult	100	23.30
Risella oil impregnated paper (control)	Adult	50	0
Cypermethrin 0.05% impregnated paper	Adult	100	46.7
Risella oil impreganated paper (control)	Adult	50	0

Susceptibility test result showed that *Ae. aegypti* population in Wonosobo was resistant toward Malathion and Cypermethrin.

Biochemical test

The results of the biochemical assay showed elevated activity of esterase (12.2%) and monooxygenase (10.4%). Elevation of enzyme activities were determeined from absorbance value exceeding the cut off point.

Table 2. Biochemical Assay of Esterase and Glutathione S-transferase Activity

Detoxifying Enzyme	N (number of examined mosquito)	% elevated enzyme activity
Esterase	90	12.2
Monooxygenase	90	10.4

Biochemical test showed that the number of mosquito with elevated esterase activity was more than the number of mosquito with elevated monooxygenase activity.

Vector Control Program by Regency Public Health Office

A survey indicated that the most common chemical used in spraying to control adult mosquito was cypermethrin.

Table 3. The Usage of Insecticides by Public Health Office in Wonosobo

Municipality	Insecticide Type	Active Ingredient	Period of Usage
Leksono	synthetic pyrethroid	cypermethrin	>5 years
Wonosobo	synthetic pyrethroid	cypermethrin	>5 years
Selomerto	synthetic pyrethroid	cypermethrin	>5 years

Table 3 showed that synthethic pyrethroid was the only insecticide type used in vector control programme by Wonosobo Health Office to control *Ae.aegypti* population. This insecticide has been used for more than 5 years in Wonosobo.

Household Insectiside

Table 4. The Usage of Household Insecticide by Society in Wonosobo

Insecticide	Active Ingredient	Insecticide Type	% of Usage			Usage Period (Years)		
			L	W	S	L	W	S
Mosquito coil	Transfluthrin 0.03%	synthetic pyrethroid	10	24	11	> 2 years	1-2 years	1-2 years
Mosquito spray	Cypermethrin 0.1%, Imiprothrin 0.31%, Prallethrin 0.03%, Prallethrin 0.2%, d-allethrin 0.15%	synthetic pyrethroid	9	4	5	1-2 years	1-2 years	1-2 years
Electric mat	D-allethrin 45 mg/mat Transfluthrin 4 mg/mat d-allethrin 40 mg/mat	synthetic pyrethroid	18	8	8	1-2 years	1-2 years	> 2 years
Liquid vaporizer	Transfluthrin 12.38 g/L	synthetic pyrethroid	2	2	4	1 year	1-2 years	1-2 year
Paper based mosquito repellant	Transfluthrin 1%	synthetic pyrethroid	0	5	2	-	< 1 year	1 year
Mosquito lotion repellant	DEET 15%	-	1	4	3	< 1 year	1-2 years	> 2 years

Note: L: Leksono; W: Wonosobo; S: Selomerto

Tabel 4 showed that mosquito coil contained synthethic pyrethroid was the most frequent insecticide type to be used in household by Wonosobo society to control *Ae.aegypti* population. This insecticide has been used for more than 1 year in Wonosobo.

DISCUSSIONS

The susceptibility test of adult mosquitoes to diagnostic concentration for malathion 0.8% impregnated paper showed a potential resistance development at 24 hours holding period. *Ae.aegypti* mosquito populations in Wonosobo are not under pressure from organophosphorus compounds for Dengue control within the last five years (see Table 3 and 4). According to interview with public health officer, Dengue control programme in Wonosobo during the last five years always done using cypermethrin for adult mosquito control. However *Ae.aegypti* population in Wonosobo showed high resistant phenomenon. We speculate that this phenomenon is related to the migrated resistant strains from other areas.

Wonosobo is new Dengue endemic area. *Ae.aegypti* population in Wonosobo might consist of various strains from other endemic areas which migrated to Wonosobo along with rising temperature caused by global warming. These migrant populations could bring malathion resistant character which came from their original habitat. Afterwards, the resistant character was more increasing with intra strain cross mating in Wonosobo. Several studies elsewhere have examined this phenomenon.[20,21] Unfortunately, data about sibling species in *Ae.aegypti* population from Wonosobo is not available.

The susceptibility test of adult mosquitoes to diagnostic concentration for cypermethrin 0.05% impregnated paper also showed a potential resistance development at 24 hours holding period. *Ae.aegypti* mosquito populations in Wonosobo are under heavy pressure from synthethic pyrethroid compounds through indoor house spraying of cypermethrin by local Public Health Office (Table 3). In addition, *Ae.aegypti* is also exposed to a range of synthethic pyrethroid compounds used in household insecticide (Table 4). Mosquito coil contained synthethic pyrethroid was the most frequent insecticide type to be used in household by Wonosobo society to control *Ae.aegypti* population. According to the questionnaire result, this insecticide has been used for more than 1 year in Wonosobo.

Mosquito coils are the most commonly used in household insecticidal product in the world with sales exceeding 45 to 50 billion coils used by two billion people worldwide each year.[22] The popularity of coils is due to their low cost, their ability to be used without electricity or equipment and cultural acceptance, because smoke is used in many cultures to drive away mosquitoes.[23] These products present a great opportunity for public health, because such products could provide a means of disease control that is already proven highly acceptable to end-users and has undergone stringent safety testing.[24]

The most frequent product found in study area was 0.03% transfluthrin mosquito coils. Transfluthrin is a highly effective fast-acting pyrethroid insecticide used extensively in household and hygiene products, mainly against flying insects, such as mosquitoes and flies. The WHO have carried out an evaluation of the extensive toxicity literature available on transfluthrin and concluded that transfluthrin is: "unlikely to present acute hazard in normal use".[25] Transfluthrin has light turbid color until browny, and easy to dilute in organic solvent such as hexane, dicloromethane and toluene, with dissolved level at 200000 mg/l. This compound is contact and stomach toxic that has better knockdown power and residue.[24]

Aedes aegypti from Wonosobo showed increased levels of esterase activity (12.2%). This percentage was lower than report form Pekalongan and Yogyakarta. This suggested that the possibility of a resistance mechanism in *Ae. aegypti* population from Wonosobo to Malathion was not only based on enzymatic reactions but also influenced by mechanisms from other pathways. However, esterases might have a role in resistance mechanism toward malathion in Wonosobo. Esterases are the detoxification enzymes involved mainly in insecticide resistance. In Malaysia, Lee had confirmed by using biochemical tests that a major factor resulting to resistance in *Cx. quinquefasciatus* was due to elevated levels of esterases which correlated directly with malathion (OP) resistance.[26] Besides this, Yu et al also had found that resistance development to malathion was highly associated with increased esterase activity which indicated that metabolic detoxification was likely the major resistance mechanism in insects other than mosquitoes such as plant bug, *Lygus lineolaris*.[27]

Monooxygenases also showed increased level in *Ae.aegypti* population in Wonosobo (10.4%). Monooxygenases (P450) are an extremely important metabolic system because of their involvement in regulating the titers of endogenous compounds such as hormones, fatty acids and steroids, and in the catabolism and anabolism of xenobiotics such as drugs, pesticides and plant toxins.[9] Similarly, P450-mediated metabolism can result in detoxification of insecticides such as pyrethroids, Hardstone stated that the high resistance to permethrin in *Culex pipiens quinquefasciatus* is due solely to P450-mediated detoxification.[28]

These metabolic enzymes involvement in insecticide resistance mechanism can cause a cross insecticide resistance in *Ae.aegypti* population in Wonosobo, especially for insecticide from the same groups or modes of action. Regular monitoring of the mosquitoes' susceptibility to the most widely used insecticides is necessary to ensure an appropriate choice of chemicals. Introduction of inappropriate insecticides without a proper understanding of the prevailing resistance mechanisms may lead to operational control failure. Moreover, early detection and knowledge of the resistance status as well as the underlying mechanisms in vector mosquitoes are essential for effective long-term control of *Ae.aegypti*. Insecticide resistance toward Malathion and Cypermethrin will be negatively impacting on the efficacy of vector control interventions in Wonosobo. To overcome this challenge, some different approaches are required in vector control strategies. Environmental management and community empowerment should be encouraged by the local government in Dengue vector control program. Hopefully, it can reduce reliance on the use of insecticides in vector control.

In conclusion, the results of the study by biochemical assay of the esterase and monooxygenase enzyme had shown a tendency to increase. The percentage of *Ae.aegypti* mosquitoes with increased enzymes activity was in line with mortality percentage of mosquito due to insecticide exposure. The number of mosquitoes with increased esterase were more than mosquitoes with increased monooxygenase. This is consistent with the percentage of dead mosquitoes due to exposure to malathion which more than cypermethrin exposure.

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