CONFERENCE PROCEEDING

2017 International Multidisciplinary Conferences on Productivity and Sustainability
Jakarta, 5-7 December 2017

Determination of *Aedes Aegypti* Resistance Status with Bioassay and Biochemical Test in Medang Tangerang against Synthetic Pyrethroid

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Control of dengue vector *Aedes aegypti* mosquito is usually done by using insecticides, whether by government or insecticides used in the household. Household insecticides commonly used are synthetic synthetic pyrethroid such as d-allethrin, transfluthrin and metofluthrin. The use of insecticides in large amounts and a long time can cause mosquito resistant. This study aims to determine the status of *Ae. aegypti* mosquito resistance in Medang Tangerang against d-allethrin, transflutrin and metoflutrin. To determine the resistance status by bioassay test method, biochemistry and susceptibility (WHO standard) by using impregnated paper containing d-allethrin 0.3%; transfluthrin 0.03% and 0.0097% metoflutrin. *Ae. aegypti* was contacted for 10, 20, 30, 40, 50 and 60 minutes. The number of mosquito deaths was calculated based on the percentage of deaths at each minute to 10, 20, 30, 40, 50 and 60 and d-allethrin, transfluthrin and metofluthrin concentrations. The results showed that d-allethrin, transfluthrin and metofluthrin insecticides had 100% killing power at 60 min and 100% susceptibility test. Based on the result of the research, it can be concluded that the duration of 100% d-alletrin, transfluthrin and metofluthrin is 100 minutes. *Ae. aegypti* mosquito is still susceptible to d-allethrin, transfluthrin and metofluthrin insecticides.

Keywords: *resistance, aedes aegypti, susceptibility test, synthetic pyrethroid*

1. Introduction

Resistance to insecticides may be functionally defined as the ability of an insect population to survive exposure to dosages of a given compound that are lethal to the majority of individuals of a susceptible lineage of the same species.[1] Resistance is based on the genetic variability of natural populations. Under insecticide selection pressure, specific phenotypes are selected and consequently increase in frequency. Resistance can result from the selection of one or more mechanisms. In order to elucidate the molecular nature of resistance, many studies report laboratory controlled selection of different species.[2-6] Furthermore, selected lineages, it becomes easier to separate the role of each distinct mechanism. In a more direct approach, the current availability of a series of molecular tools enables detection of expression of altered molecules in model organisms so that the effect of the insecticide can be evaluated under specific and controlled circumstances.[7]

In this research will be tested by bioassay and biochemical resistance to determine the activity of esterase enzyme related to mechanism of mosquito resistance vector against synthetic insecticide pyretroid d-alletrin 0.3%, transfluthrin 0.03% and metofluthrin 0.0097%. In this study used insecticides type like d-allethrin 0.3%, transfluthrin 0.03% and metofluthrin 0.0097% because the most widely used by the community. Compared with bioassays, biochemical resistance test results can be obtained more rapidly so as to detect the occurrence of resistance early, and can to determine the possibility of cross reactions with insecticides.[8] Determination of mosquito resistance status of DHF vectors periodically is necessary to obtain further detection base data and monitoring the occurrence of resistance. Thus the potential characteristics of the occurrence of resistance can be known earlier for consideration in the vector control strategy. This study was conducted to determine the occurrence of *Ae. aegypti* mosquito resistance due to continuous use of synthetic mosquito repellent. If there is resistance then the next step is to replace the type

of synthetic mosquito repellent that is more effective against *Ae. aegypti* mosquito in order not to cause damage to the environment and the health of its users.

2. Experimental Disease

This research is an observational research. The research will be conducted by taking mosquito samples from Medang Tangerang area. Implementation of bioassay and biochemical tests conducted at Entomology Laboratory of Faculty of Medicine Ukrida. The population of this study was *Ae. aegypti* adult larvae culture obtained in the field allegedly inherited the nature of its parent resistance. The sample of this study was taken randomly as many as 25 *Ae. aegypti* mosquitoes adult per unit of observation.[9] The mosquitoes used were colonized from the study site with a stomach full of blood (healthy) condition, then prepared 4-5 WHO standard test tubes and on the tubes with red marks inserted insecticide paper (d-allethrin 0.3%; transfluthrin 0.03% and metofluthrin 0.0097%). In this study used 25 female mosquitoes with satiety condition of blood then put into test tube of red mark and exposed with insecticide for 0, 10, 20, 30, 40, 50 and 60 minutes. A total of 25 female mosquitoes as a control were inserted into a tube containing green markers and equipped with paper without insecticide (solvent). After the mosquito is exposed to the insecticide then transferred to the holding tube with a wet towel. The bioassay test is counting the number of mosquitoes died due to exposure to synthetic mosquitoes at minute 0, 10, 20, 30, 40, 50 and 60. Data on the number of mortality obtained will be calculated as knockdown time 50 (KT_{50}) and knockdown time 90 (KT_{90}). By calculating KT_{50} and KT_{90} it will get the value of Estimated Resistance Ratio (ERR) that determines the level of mosquito resistance. Criteria: death <80% is resistant, deaths 80-98% are tolerant and deaths 99-100% are vulnerable.[10] The test should be repeated if there is a death in the control group of more than 20%. The death of mosquito is corrected by the Abbot formula (WHO standard). The value of resistance ratio is obtained from the calculation of the percentage of death rate of the test mosquitoes by treatment and compared with the still-vulnerable mosquitoes (control). The biochemical test is measuring the level of esterase enzyme activity, one of which is the activity of acetylcholinesterase associated with paralysis in mosquitoes.

3. Results and Discussion

Research data was obtained by calculating the mortality rate of test and control mosquitoes after exposure d-allethrin 0.3%; transfluthrin 0.03% and metofluthrin 0.0097% over 10, 20, 30, 40, 50 and 60 min. Of the 25 mosquitoes tested, the percentage of mosquitoes that were knockdown and during holding and while exposure of insecticides were observed. Observation data can be seen in Figure 1. KT_{50} and KT_{90} of *Ae. aegypti* mosquito in Medang Tangerang tested with probit analysis. The probit analysis shown in Table 1.

Table 1. Estimated Resistance Ratio, KT_{50} and KT_{90} against Piretroid Synthetic Insecticides

No	Insecticide	KdT_{50}	ERR	KdT_{90}	ERR
1	d-allethrin 0.3%	30.16	1.01	49.63	1.02
2	Transfluthrin 0.03%	34.22	1.15	52.66	1.08
3	Metofluthrin 0.0097%	39.04	1.31	60.08	1.23
4	Laboratorium	29.75	-	48.80	-

Test susceptibility for 60 min, showing the mortality rate of *Ae. aegypti* mosquitoes dies entirely (100%) in the d-allethrin 0.3% treatment group and transfluthrin 0.03%, whereas in the metofluthrin 0.0097% treatment group mortality was only 90%. The results of calculation of resistance ratio value (ERR) for d-allethrin 0.3%, transfluthrin 0.03% and metofluthrin 0.0097% showed less than 10 (ERR <10), meaning that the test of mosquitoes are still vulnerable. The nature of resistance that emerges in the early minutes may occur as one of the effects of persistent insecticide use by both government and society. The occurrence of resistance is influenced by several factors, especially the use of insecticides for a long time (about 2 - 20 years), and non-standard doses.[11] According to WHO, there are three basic mechanisms that play a role in the process of resistance insecticide susceptibility to insecticides, including: increased insecticide toxic metabolism in insecticide with mixed function oxidase, hydrolase, esterase like acetylcholinesterase enzymes; changes in the sensitivity of the insides of insect bodies, in the form of nerve

insensitivity and intensity of the enzyme acetylcholinesterase (AChE); decreased toxic penetration (insecticide) to the active site (nerve and AChE) (WHO, 1980).[12] Biochemical test on *Ae. aegypti* showed an increase in the activity of acetylcholinesterase enzyme in pyrethroid insecticide group (d-allethrin, transfluthrin and metofluthrin) compared to control, as seen in Figure 2.

Synthetic pyretroid insecticide (d-allethrin, transflutrin and metoflutrhin) has a way of inhibiting the enzyme acetylcholinesterase, causing disruption to nerve activity due to accumulation of acetylcholine on nerve endings. The function of the acetylcholinesterase enzyme is to hydrolyze acetylcholine into choline and vinegar, so that when the enzyme is inhibited the hydrolysis of acetylcholine does not occur so that the muscle will remain contracted for a long time it will occur spasticity.[13-14] At the nerve endings of the insect nervous system will be produced acetylcholine when the nerve gets stimulation or stimulation. Acetylcholine acts as a mediator or intermediate between the nerves and muscles of the flesh allowing electrical impulses that stimulate the muscle of the flesh to contract. After the contraction period is complete, acetylcholine is destroyed by acetylcholinesterase enzyme into choline, lactate and water. When acetyl cholinesterase not immediately destroyed then the muscle will remain contracted in a long time so that there will be spasms or convulsions. By using a mosquito-fueled drug made of pyrethrin, the activity of cetylcholinesterase enzyme is bound or crushed so that muscle spasms continue (paralysis), and the mosquitoes will eventually die.

4. Conclusion

Resistance ratio values of bioassay, biochemical and susceptibility test results using d-allethrin 0.3%; transfluthrin 0.03% and metofluthrin 0.0097% are still susceptible to *Ae. aegypti* mosquitoes which can also be seen from ERR <10.

References and Notes

1. B.J. Beaty, and W.C. Marquardt. University Press of Colorado, Colorado **(1996)**.
2. C. Chang, W.K. Shen, T.T. Wang, Y.H. Lin, E.L. Hsu, and S.M. Dai. *Insect Biochem Mol Biol*. 39(4), 272-278 **(2009)**.
3. S. Kumar, A. Thomas, A. Sahgal, A. Verma, T. Samuel, and M.K. Pillai. *Arch Insect Biochem Physiol*. 50(1),1-8 **(2002)**.
4. P. Paeoporn, N. Komalamisra, V. Deesin, Y. Rongsriyam, Y. Eshita, and S. Thongrungkiat. *Southeast Asian J Trop Med Public Health*. 34, 786-792 **(2003)**.
5. M.M. Rodriguez, J.A. Bisset, C. Diaz, and L.A. Soca. *Rev Cubana Med Trop*. 55(2), 105-111 **(2003)**.
6. K. Saavedra-Rodriguez, L. Urdaneta-Marquez, S. Rajatileka, M. Moulton, A.E. Flores, I. Fernandez-Salas, J. Bisset, M. Rodriguez, P.J. McCall, M.J. Donnelly. *Insect Mol Biol*. 16(6), 785-798 **(2007)**.
7. T.J. Smith, S.H. Lee, P.J. Ingles, D.C. Knipple, and D.M. Soderlund. *Insect Biochem Mol Biol*. 27(10), 807-812 **(1997)**.
8. K. Lidia, dan E.L.S. Setianingrum. MKM. 03(02), 105-110 **(2008)**.
9. P. Herath. WHO, Geneva. **(1997)**.
10. World Health Organization. WHO, Geneva. **(1975)**.
11. G.P. Georghi. Plenum Press, New York **(1983)**.
12. World Health Organization. WHO, Geneva 82p. **(1980)**.
13. H. Perumalsamn. *Journal of Medical Entomology* 46(6):1420-1423 **(2009)**.
14. R.D. Ndione, O. Faye, M. Ndiaye, A. Dieye, and J.M. Afoutou. *African Journal of Biotechnology* 6(24): 2846-2854 **(2007)**.

Figure Captions

Figure 1. The percentage of *Ae. aegypti* mortality against exposure to synthetic pyrethroid insecticides. (a) 0, (b) 10, (c) 20, (d) 30, (e) 40, (f) 50 and (g) 60 is the observed time of mosquito mortality

Figure 2. The average activity of acetylcholinesterase enzyme in *Ae. aegypti* group of synthetic pyrethroid insecticide treatment. (d) d-allethrin, (t) transfluthrin, (m) metofluthrin

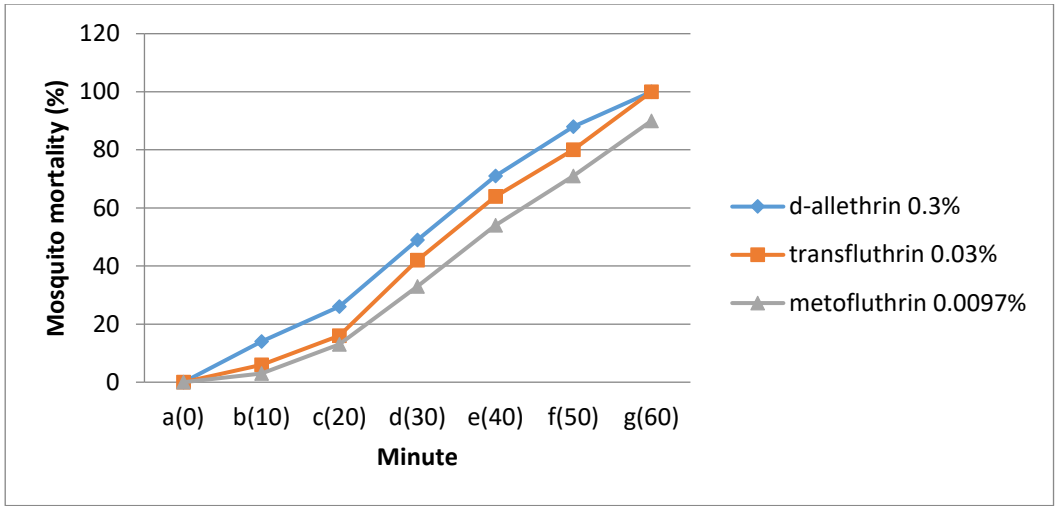

Figure 1. Susilowati et al.

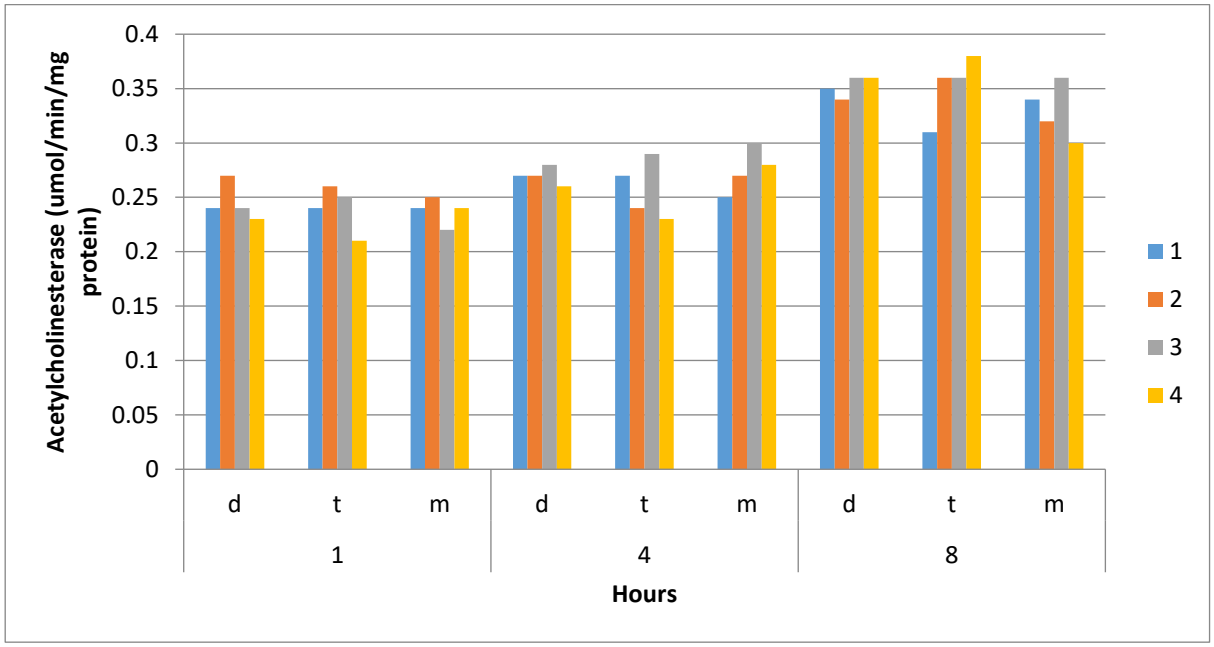

Figure 2. Susilowati et al.