Detecting Conventional Resistence of aedes aegypti in kediri disgtrict east java indonesia

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Detecting Conventional Resistance of Aedes Aegypti in Kediri District, East Java, Indonesia

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ABSTRACT

Kediri District is one of the Districts in East Java Province, Indonesia, that is categorized as an area with Extraordinary Occurrence for Dengue Fever. In 2015, it was occurred double increase for dengue fever cases rather than in 2014. The distribution of domicile for Dengue Hemorrhagic Fever sufferers in Kediri District in 2016 was mostly in Pare Subdistrict and the sufferers were 107 sufferers. Then, the next subdistrict was Ngasem Subdistrict, which was 90 sufferers, and Kunjang Subdistrict, which was 74 sufferers. Vector control of Dengue Hemorrhagic Fever for a stadium of adult mosquito uses active ingredient of Malathion. Resistance of vector against insecticide is a global phenomenon and an obstacle to the success of vector control chemically. This research aimed at analyzing detection conventionally regarding the resistance of Aedes aegypti mosquito as Dengue Hemorrhagic Fever vector in Kediri District against Malathion. This research was true experimental research and the procedures of examination in this research used WHO standard of Susceptibility test through using impregnated paper with Malathion dose in 0,8%, 0,5 %, and 0% (control variable). Data analysis referred to resistance status from WHO standard and it analyzed the difference of experimental biota death by using the statistic of ANOVA difference test. Research results showed that *Aedes aegypti* mosquito in Kediri District was resistant against malathion 0,8%, meanwhile, the use of malathion 5% was in the category of tolerance of 60 minutes. There was a significant influence between contact time and death of the *Aedes aegypti* mosquito. Suggestion for Health Office in Kediri District was the use of malathion as insecticide needed concentration in more than 0,5%.

1. INTRODUCTION

Dengue hemorrhagic fever has become a worldwide problem [1]. Indonesia has DHF cases with the first rank based on the incidence (IR) and case fatality rate (CFR) [2]. The 2016 DHF incidence rate increased by 77.96/100,000 population compared to the previous year [3]. In the world, DENV infection had occurred in Bangkok and patients were also isolated in 1958 [4]. After the incident in Bangkok, dengue fever occurred in several countries such as Cambodia, China, India, Indonesia, Malaysia, Myanmar, Singapore, and several

Pacific islands. Until now the mechanism and pathogenesis of DHF are still not fully understood. Some of the risk factors studied included viral virulence, [5] increased immunity, [6] cytokine storms, [7] lipid profile changes, [8] autoimmune responses, [9] host genetic factors, [10] bacteremias caused by Staphylococcus aureus [11], [12]. The control of dengue hemorrhagic fever has been comprehensively implemented in Indonesia since 1968 [13]. The programs implemented include adult perifocal spraying, mass larvicides, and disease control counselling to the public. However, cases of dengue fever have developed and become hyperendemic. Several large cases have been reported in Indonesia [14- 17].

Since the first dengue reports in Jakarta and Surabaya in 1968, the epidemiology of dengue in Indonesia has changed [18]. According to data from Health Office in East Java Province in 2015, DHF sufferers in 38 cities were 20.707 sufferers and 284 sufferers died. Meanwhile, in 2016, the total of DHF sufferers were 20.639 sufferers and 298 sufferers died. Hence, the level of Case Fatality Rate (CFR) reached 1,4 per cent.

Until recently, there has been no effective way to overcome DHF disease because dengue anti-virus drugs have not been found, then, it is a needed way to prevent dengue fever by reducing the population density of Aedes aegypti mosquitoes to the lowest possible, which is through mosquito population control.

Among 38 Districts/Cities in East Java Province, Kediri District is one of the Districts which is categorized as an area with Extraordinary Occurrence for Dengue Fever because, in 2015, it was occurred a double increase for dengue fever cases rather than in 2014. The distribution of domicile for Dengue Hemorrhagic Fever sufferers in Kediri District in 2016 was mostly in Pare Subdistrict and the sufferers were 107 sufferers. Then, the next subdistrict was Ngasem Subdistrict, which was 90 sufferers, and Kunjang Subdistrict, which was 74 sufferers [19].

Controlling vectors chemically through using insecticide for adult mosquitoes will stimulate the occurrence of selection against the population of insects that becomes the target. Intensive and uncontrolled use even inappropriate use with the user guide will cause quickly the resistance for the insect against insecticide. As the impact, if the insect has been resistant, the failure of controlling will occur [20].

Controlling DHF vector for a stadium of adult mosquito is through fogging which uses active ingredient of Malathion. The active ingredient is an insecticide in the organophosphate type. The insecticide is used in Indonesia since 1970, as well as in Kediri District. However, until recently, DHF cases are still many, thus, it will enable to occur an extraordinary occurrence for dengue fever. This condition causes a question whether it has occurred a resistance for Aedes aegypti mosquito as DHF vector against Malathion or not.

Early detection for the resistance of vector against insecticide can be used as an information program for selecting proper insecticide in controlling vector locally specific in decentralization era. Detection of vector resistance against insecticide can be conducted in several ways: Conventionally detection by using WHO standard method in susceptibility test through using impregnated paper, Biochemical or enzymatic detection by using microplate, and Molecular detection.

This research aimed at detecting conventionally the resistance of Aedes aegypti mosquito as DHF vector in Kediri District against Malathion.

2. Materials and Methods

This research was true experimental research. Sample in this research was 3rd generation (F3) of *Aedes aegypti* mosquito stadium that was bred in the laboratory from parental which was from an egg that was

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trapped into ovivitrap in an endemic area of DHF in Pare Subdistrict, Ngasem Subdistrict, Kandat Subdistrict, and Kunjang Subdistrict, Kediri District, East Java Province, Indonesia.

The procedure of breeding mosquitoes is that eggs are obtained from the field and placed in a plastic tray filled with dug well water, let stand until the eggs hatch into larvae, Raise a larva until to become a pupa and feed it with dog pellet powder. The pupae formed is then transferred to a plastic container and placed in a mosquito cage waiting to turn into an adult mosquito (referred to as F1) bringing up adult mosquitoes by feeding a 10% sugar solution and blood feed of guinea pigs placed in clamp cages for graphite females (contains eggs). The process is continued until we get F2 and F3.

Meanwhile, the procedures of examination in this research used the WHO standard of susceptibility test through using impregnated paper with Malathion dose in 0,8%, 0,5 %, while for control using Rosella oil-impregnated paper. test tube with red mark as exposure tube, tube with a green mark as the holding tube. Data analysis in this research referred to resistance status by referring to the standard category from WHO (21)

3. Findings

Result of conventional resistance test against Malathion 0,8% and 5% against Aedes aegypti mosquito with contact time in 15 minutes, 30 minutes, 45 minutes, 60 minutes in 3 (three) repetitions.

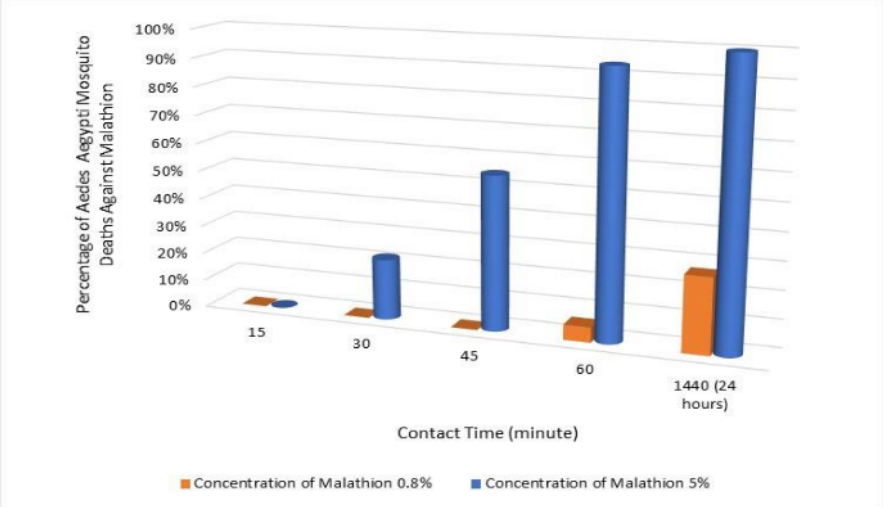

Chart 1

Percentage of *Aedes Aegypti* Mosquito Deaths Against Malathion In Kediri District

From Chart 1 above, it was obtained that Conventional Resistance Test exposed the 20 *Aedes aegypti* mosquitoes which were from Kediri District for 60 minutes by using Malathion 5%. Every 15 minutes, was conducted an observation. The result that was obtained in the first 15 minutes of contact time showed that there was no dead *Aedes aegypti* mosquito. The death of the *Aedes aegypti* mosquito started in 30th, 45th, and 60th minutes with an average percentage in *Aedes aegypti* mosquito death in Kediri District as much as 21,67%; 55,00%; 93,75 %. Observation of *Aedes aegypti* mosquito death in contact time for 24 hours (1440 minutes) that was caused by the residue of malathion 5% was obtained death percentage in 100% as well as with the use of malathion 0,8% in resistance test that exposed 20 *Aedes aegypti* mosquitoes for 60 minutes. Moreover, it was obtained that there was no dead mosquito after having contact time for 15, 30, and 45 minutes, but in 60th minutes, the *Aedes aegypti* mosquito died in 5,42%. Besides, the average death

percentage increased to be 27,08%, which was caused by the residue of malathion 0,8% for 24 hours (1440 minutes). Meanwhile, in the control group, there was no mosquito death.

Table 1 Resistance Status of *Aedes Aegypti* Mosquito Against Malathion In Kediri District

Contact Time (minute)	Resistance Status of *Aedes Aegypti* Mosquito	
	Concentration of Malathion	
	5%	0,8%
15	Resistant	Resistant
30	Resistant	Resistant
45	Resistant	Resistant
60	Tolerant	Resistant
1440 (24 hours)	Susceptible	Resistant

From Table 1 above, it was obtained that the *Aedes aegypti* mosquito from Kediri District was resistant against malathion 0,8% which started from the first 15 minutes, but the *Aedes aegypti* mosquito was resistant with malathion 5% after being exposed for 15, 30, and 45 minutes. Resistance Status of *Aedes aegypti* mosquito changed to be tolerant in 60th minutes and changed to be susceptible due to the impact from the residue of malathion 5% during 24 hours (1440 minutes), but *Aedes aegypti* mosquito was resistant against malathion 0,8%.

Table 2 Anova 2 for the Direction of Mosquito

Variation Source	Free degrees	Total of Squares	Middle Squares	F Count	P
Corrected Model	9	3618.232[a]	402.026	3.049	.010
Intercept	1	2998.612	2998.612	22.745	.000
Time	*4*	*2882.589*	*720.647*	*5.466*	*.002*
Malathion	1	186.408	186.408	1.414	.244
time * of malathion	4	549.234	137.309	1.042	.402
Error	30	3955.012	131.834		
Total	40	10571.856			
Corrected Total	39	7573.244			

From Table 2 above, it could be obtained that there was a significant influence between contact time for 15, 30, 45, and 60 minutes against the average of mosquito death ($P < \alpha = 0.05$), meanwhile, the concentration of malathion and interaction of time did not have any significant influence against mosquito death ($P > \alpha = 0.05$). Besides, there was a significant difference between contact time for 15 – 60 minutes, 15, 30, 45 minutes and 24 hours against the average of mosquito death. (Looked at Table 5.9). On the other hand, there was no significant difference in the average of mosquito death between the Districts ($P > \alpha$).

4. Discussion

Regarding the death of *Aedes aegypti* mosquitoes, using malathion type in 0,8% was ineffective to eradicate *Aedes aegypti* mosquitoes mostly and in the 60th minutes, the mosquito death was in the average of percentage in 5,42%. Being seen from each sub-district, only in Kandat Sub-district was the place where *Aedes aegypti* mosquito died, meanwhile, for other sub-districts until in the 60th minutes, there was no dead

Aedes aegypti mosquito. This was due to too low a concentration of insecticide, thus, there were still many live *Aedes aegypti* mosquitoes. The death of *Aedes aegypti* mosquitoes through using insecticide of malathion with a concentration of 5% greater, the *Aedes aegypti* mosquitoes died with an average of percentage in 93,75% more in the concentration of 0,8 %. Moreover, it was following conducted research which showed that resistance test through using impregnated paper that had active ingredients of Malathion insecticide in 0,8% was obtained the result that among eleven areas which were researched, all of them were resistant with *Aedes Aegypti* mosquito death and it was around 0 – 14% [22].

Susceptibility test for *Aedes aegypti* mosquitoes from Kediri District gave resistant result against malathion 0,8%, meanwhile, susceptibility test by using malathion insecticide in 5% was resistant until the 45th minutes. However, in 60th minutes, the resistance status of the *Aedes aegypti* mosquito changed to be tolerant and to be susceptible in 24 hours of mosquito death due to the residue of malathion 5%. Furthermore, almost all status of *Aedes aegypti* in an area that had the highest DHF case was in resistant status. This was occurred due to the impact of the mosquito control program through using insecticide in malathion type continuously. This showed that the more often fogging was done in an area by using malathion, the greater the mosquito's immunity against malathion and the immunity would be inherited in the next offspring. It stated that insecticide by using chemical material would cause stress for the mosquito, hence, the mosquito would adapt to maintain life on the insecticides even at different doses [21]. Ae. Aegypti resistance can occur due to repeated chemical application or application of two or more insecticides with different mechanisms [23].

The research that is conducted by [24] in Banten Province, namely Cilegon, Serang City and Tangerang City, shows the same results that *Aedes aegypti* mosquitoes have experienced continuous exposure without rotation and errors in using insecticides resulting in mosquitoes experiencing resistant. *Aedes aegypti* mosquito in Buah Batu District, Bandung City is also resistant to malathion insecticide 0.8% [25]. *Aedes aegypti* mosquitoes in Tangerang City also experienced resistance to the results of malathion research [26].

Contact time influenced significantly the death of the *Aedes aegypti* mosquito ($P < \alpha$), but the concentration of malathion and interaction of malathion with contact time did not have any significant influence on the death of the *Aedes aegypti* mosquito ($P > \alpha$). In concentration of malathion 0,8 % with the longer contact time, which was 60 minutes, the *Aedes aegypti* mosquitoes were still resistant, thus, Malathion for eradicating *Aedes aegypti* mosquitoes could not be used in low concentration again, meanwhile, in concentration 5%, the mosquitoes were in non-resistant condition but it was needed longer exposure time between mosquito and malathion. Besides, it also could be conducted combination through giving higher than 5% in the concentration of malathion with the shorter contact time, which was less than 60 minutes. The result of this research was in line with the result of resistance examination against *Aedes aegypti* mosquito that showed all of the mosquitoes which were the sample here were examined and the mosquitoes had been resistant against malathion 0,8%. The result of the mosquito death in this examination was found 0% [20]. The process of the occurrence of resistance in several insects, including mosquitoes, were influenced by 3 factors, which were 1) genetic factor [27]. There were several genes, which had a role in controlling either dominant resistance or recessive resistance, such as genes that formed esterase enzyme which could cause an insect to be resistant against organophosphate and pyrethroid insecticides. 2) Biological factors, such as there was a change of generation, marriage and behaviour such as migration and morphological isolation. 3) Operational factors, such as chemical material that was used and application of insecticide use in the field, for example, application method, frequency and duration of use.

The use of malathion in the long term is the cause of the *Aedes aegypti* mosquito resistance, this is evidenced from his research that examined in the area of DKI Jakarta, where DKI Jakarta has used malathion insecticide

since 1970. The organophosphate insecticides have a mechanism of action inhibiting the action of the enzyme acetylcholine esterase in mosquitoes [28]. The increase in esterase enzyme is in line with the percentage of mosquito mortality, this shows that the resistance of mosquitoes to the malathion insecticide is based on metabolic resistance mechanism. Malathion insecticide can immobilize insects by attacking the synapses' nerves. The resistance will arise if only one type of insecticide is used and carried out continuously for a long period (1). Based on the results of Sunaryo's research in 2014 The resistance of the *Aedes aegypti* mosquito can occur by looking at the activity of the esterase enzyme in the mosquito's body being detected very high [29].

5. Conclusion

The average percentage for the death of *Aedes aegypti* mosquitoes in Kediri District which were exposed by Malathion 0,8% and 5% in contact time for 60 minutes was 5,42% and 93,75%. *Aedes aegypti* mosquitoes in Kediri District were resistant against malathion 0,8%, meanwhile, the use of malathion 5% was in a tolerant category in contact time for 60 minutes. In other words, there was a significant influence between contact time and *Aedes aegypti* mosquito death.

For suggestion from the researchers in this research was the use of malathion as insecticide needed in greater concentration, which was more than 0,5%. Fogging through using malathion should be conducted continuously and evaluated every time. It needed a resistance test for the mosquito by using other insecticide materials, which was other than organophosphate. However, it needed an observe and monitor the vector resistance continuously in every different area. the process of mixing Malthionic insecticides and the method of spraying must be in accordance with established procedures.

Author Contribution

All Authors Builds Concepts, Designs, And Searches For Literature Searches, While Data Collection, Manuscript Review, And Manuscript Finalization.

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Conflict Of Interest

Authors Declare That There Is No Conflict of Interest

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