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**Spatial Distribution Of The Conventional Resistance Of Aedes
Aegypti Mosquito And Case Of Dengue Fever, Kediri,
Indonesia, 2020**

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Abstract

Dengue Hemorrhagic Fever (DHF) is a health problem in the world. The highest number of DHF sufferers in Kediri Regency in 2016 was in the Districts of Pare, Ngasem and Kuning. DHF vector control techniques include: fogging using the active ingredient malathion for the adult stage of mosquitoes. Vector resistance to insecticides is a global phenomenon, especially for vector-borne disease control program managers and is the single obstacle to the success of chemical vector control. Detection of vector resistance using conventional detection with the standard method of WHO Susceptibility test using impregnated paper. The purpose of the study was to describe the Spatial Distribution of Conventional Resistance of *Aedes aegypti* Mosquitoes in Kediri Regency. This type of True Experiment research uses *Aedes aegypti* mosquitoes in dengue-endemic areas in Kediri Regency. The research sample was the 3rd offspring (F3) of the adult *Aedes aegypti* mosquito. Exposing the *Aedes aegypti* mosquito with 5% malathion insecticide with a contact time of 45 and 60 minutes. Data analysis includes the percentage of mosquito mortality, determining resistance status by referring to the WHO category standard and describing mosquito resistance and connecting the percentage of mosquito deaths with dengue fever cases using the *geoda* application. samples of the test biota (*Aedes aegypti* mosquito larvae) were obtained from 11 (eleven) sub-districts, namely Pare, Ngasem, Kandat, Kuning, Purwoasri, Wates, Gampeng, Papar, Puncu, Kayen, Kandangan. The mortality percentage of *Aedes aegypti* mosquitoes exposed to Malathion 5% with exposure within 60 minutes is less than 90%, namely Pare District with resistant status, while ten (10) other districts are above 90% with vulnerable status. The percentage of sub-districts that use malathion insecticides for more than 10 years is 54.54% and the use of malathion insecticides following the procedures set by the government. It is necessary to rotate the use of insecticides in controlling the DHF vector, monitoring and evaluating the susceptibility status of the DHF vector to insecticides used by the Kediri District Health Office every 1-2 years, as well as monitoring and evaluating the density of mosquitoes.

Keywords : resistensi, *Aedes aegypti*, malathion

Introduction

In 2016 in Kediri Regency there were 993 cases of dengue fever (IR = 64.19/100,000 population) with 18 deaths (CFR = 1.8%). Compared to the number of cases in 2015 there was a very sharp increase, the number of dengue cases was 702 people with 7 deaths. Kediri District Health Office, 2016) and extraordinary events occurred in 2019, with a population of 1,577,623 people obtained IR = 121.26/1,577,623 residents with 27 deaths (CFR = 2.79%)(Kediri, 2016)

The DHF vector control technique carried out in Kediri Regency is fogging for adult stage mosquitoes using the active ingredient malathion and the mosquito larvae stage using the active ingredient temephos. The active ingredient used is an organophosphate group insecticide. The same study was also conducted in Pekalongan Regency, using the insecticide malathion with a concentration of 6%

within 60 minutes to kill *Aedes aegypti* mosquitoes with a mortality percentage of 100%. (Sudaryanto, 2010)

Aedes aegypti mosquitoes in Kediri Regency, especially in Ngasem and Kandat Subdistricts are susceptible to 5% malathion insecticide with exposure in approximately 60 minutes, while in Pare and Kunjang Subdistricts which have dengue cases have experienced susceptibility to 5% malathion insecticide. This shows that the more often an area is fogged using malathion, the greater the immunity of mosquitoes to malathion and the immunity will be passed on to the next generation. According to Firda, in her research, she stated that 3 factors greatly influence mosquito susceptibility, namely genetic, biological and operational factors (Pradani et al., 2011).

Research in 2018 proved that the *Aedes aegypti* mosquito in Kediri Regency in 4 (four) sub-districts namely Pare, Ngasem, Kandat and Kunjang Districts was resistant to 0.8% malathion, while the use of 5% malathion was in the tolerant category within 60 minutes of exposure. With the percentage of *Aedes aegypti* mosquitoes exposed to Malathion 0.8% and 5%, 5.42% and 93.75% respectively. Resistance to *Aedes aegypti* is caused by excessive and continuous administration of malathion with the same concentration. (Marlik et al., 2018).

The process of decreasing susceptibility to some insects including mosquitoes can be influenced by 3 factors, namely genetic factors, biological factors and operational factors, in this case, the characteristics of the chemicals used and the application of these insecticides in the field. This study will map the distribution of *Aedes aegypti* mosquito resistance based on the application method, frequency of use and duration of use of malathion insecticide in each district of Kediri Regency.

Materials and Method

This research is descriptive. The study was carried out by taking samples of *Aedes aegypti* mosquito larvae from Kediri Regency in sub-districts that have endemic villages based on data from the Kediri Regency Health Office. The bioassay test (the standard method of WHO Susceptibility test using impregnated paper) was carried out in the Entomology Laboratory of the Environmental Health Department, Poltekkes, Ministry of Health, Surabaya. Create resistance mapping using ArcView and good programs.

The object of this research is the 3rd generation (F3) larval and adult stages of the *Aedes aegypti* mosquito breeding in the entomology laboratory of the Environmental Health Department, Poltekkes, Ministry of Health, Surabaya, from parental eggs trapped in ovitraps in dengue-endemic areas in Kediri Regency. Provide 3 tubes with red dots. In each tube, impregnated paper (paper with the Malathion insecticide) is inserted, then 20 F3 *Aedes aegypti* mosquitoes are inserted into the test tube, fully fed, and exposed for 60 minutes. For control, provide 2 test tubes with green spots and insert paper that does not contain an insecticide, then add 20 mosquitoes with fully fed stomach conditions, during exposure the temperature and humidity are recorded, after the test and control mosquitoes are exposed, then the mosquitoes are transferred to paper cups, and allowed to be stored for 24 hours, during storage the temperature and humidity were recorded, so that during storage the mosquitoes did not die, then given a wet towel for 24 hours storage, the test results were recorded on the observation sheet.

RESULTS

Resistance and Mortality Percentage of *Aedes aegypti* Mosquitoes exposed to Malathion 5%

Mosquito biota sampling was collected from the sub-districts of Purwoasri, Wates, Gampeng, Papar, Puncu, Kayen, Kandangan, Pare, Ngasem, Kandat and Kunjang. Samples were taken directly from residents' homes in the form of larvae in a water reservoir as a sample unit. The samples obtained were put in plastic bottles and taken to the entomology laboratory of the Department of Environmental Health Poltekkes, Ministry of Health, Surabaya, where they were cultured to become adult mosquitoes of F3 offspring.

Aedes aegypti mosquito larvae originating from the research site were bred at the Surabaya Environmental Health Entomology Laboratory. The conventional resistance test using the standard method of the WHO Susceptibility test using impregnated paper was carried out according to the procedure and was observed to produce mosquito mortality data as follows:

Table 1
Percentage Of *Aedes Aegypti* Mosquito Death
Against 5% Malathion

No	Sub districts	Time				
		15 s	30 s	45 s	60 s	24 hours
1	Pare	0	0	15	78.33	100
2	Ngasem	0	11.67	28.33	98.33	100
3	Kandat	0	71.67	95	100	100
4	Kunjang	0	3.33	81.67	98.33	100
5	Purwoasri	15	51.67	90	100	100
6	Wates	20	60	95	100	100
7	Gampeng	15	68.33	100	100	100
8	Papar	20	50	88.33	100	100
9	Puncu	15	55	98.33	100	100
10	Kayen	23.33	56.67	98.33	100	100
11	Kandangan	32.5	71.67	100	100	100

A map of the resistance distribution showing the level of susceptibility of the *Aedes aegypti* mosquito at the 60th minute which is determined from the status of resistance or insecticide susceptibility to insects from the results of the *Aedes aegypti* mosquito mortality data in Table 1 which was measured using a standard susceptibility test procedure.

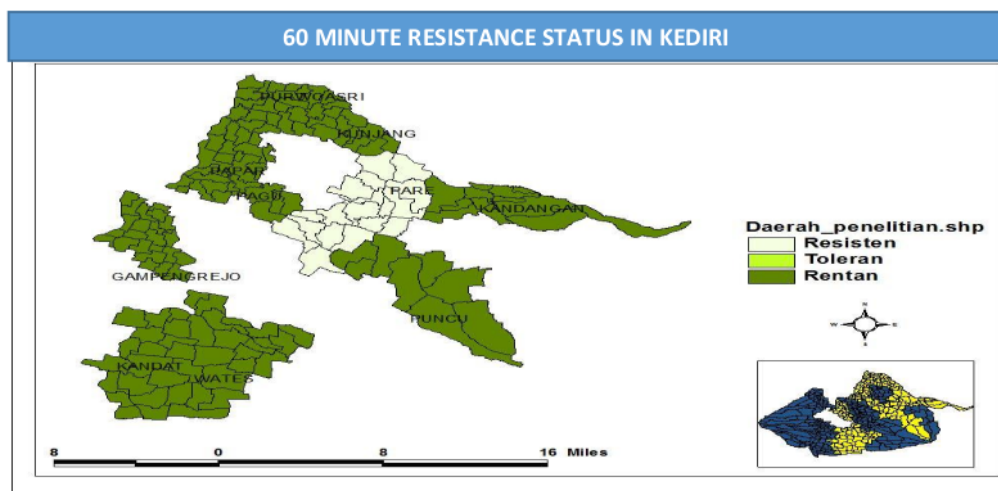


Figure 1 Distribution Map of Resistance Status at 60 Minutes in Eleven (11) Districts of Kediri Regency in 2020

The results of exposure to organophosphate insecticides of the malathion type in *Aedes aegypti* mosquitoes in this study gave results from 11 (eleven) Districts, there was 1 (one) District of *Aedes aegypti* mosquito mortality less than 90%, namely Pare District, while ten (10) *Aedes* mosquito mortality districts *aegypti* above 90% at 60 minutes.

Characteristics of the use of malathion insecticides in 11 (eleven) sub-districts

The results showed that the use of insecticides in the effort to eradicate dengue vectors was Malathion insecticide, from 11 sub-districts all of them used malathion. The use of malathion in adult mosquito control which is applied with a Fogging tool is following operational procedures by mixing diesel fuel. the frequency of spraying is according to the number of cases, from 11 sub-districts there are 54.5% using more than 10 years

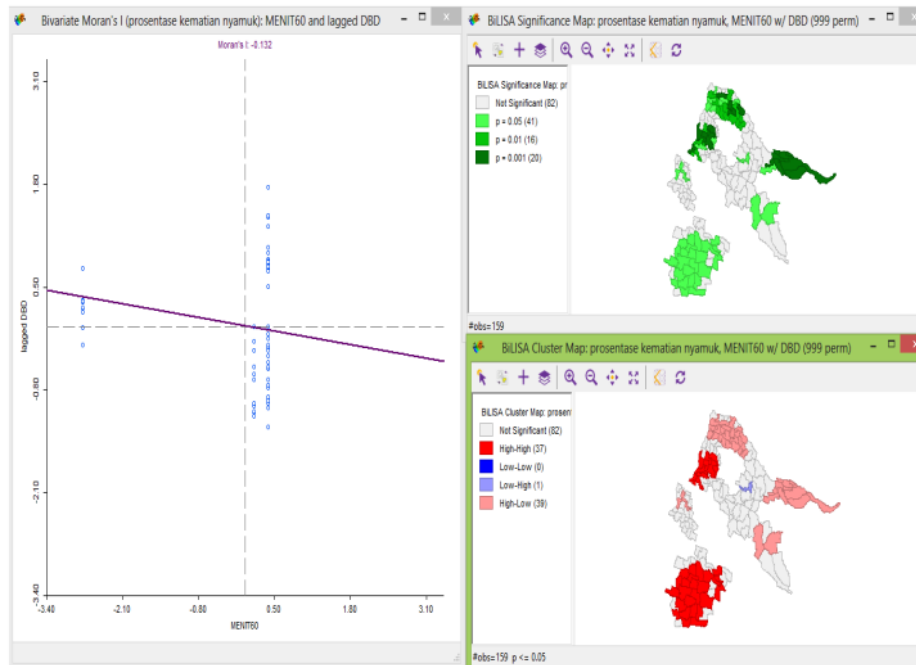
Number of Dengue Fever Cases in 11 (Eleven) Sub-Districts

The number of dengue fever cases in 2019 in Pare sub-district was 81 cases, Ngasem sub-district as many as 56 cases, Kandat sub-district as many as 85 cases, Kunjang sub-district as many as 18 cases, Purwoasri sub-district as many as 26 cases, Wates sub-district as many as 81 cases, Gampeng sub-district as many as 26 cases, Papar District has 16 cases, Puncu District has 25 cases, Kayen District has 31 cases and Kandangan District has 13 cases. (Kediri D. K., 2019).

Map of the relationship between *Aedes aegypti* mosquito resistance in Malathion and Dengue Fever Cases in 2019 in 11 (Eleven) Sub-Districts

The illustration of the resistance distribution map below shows the relationship between the percentage of mosquito deaths and the number of dengue cases in Kediri district, which can be seen below.

Figure 2 Relationship between the percentage of *Aedes aegypti* mosquito deaths in ion and Dengue Fever Cases in 2019 in



Eleven (11) sub-Districts

The distribution of the percentage of mosquito deaths to dengue fever cases, using the geoda application and the Bilisa test, is divided into 4 (four) clusters, namely high - high, meaning that the percentage of mosquito mortality is high/susceptible mosquitoes, but also high dengue cases, as for those included in the cluster are sub-districts. kandat, Wates, Papar and Pagu. Cluster low – high is the percentage of small mosquito deaths/mosquito status in the resistant category, while for cases of high dengue fever, the sub-district that is included in the cluster is Pare District. The last cluster is High – Low, where the percentage of mosquito mortality is high/mosquito status, is in the vulnerable category while cases of dengue fever are high.

The relationship between the percentage of mosquito mortality and the number of dengue cases, with a significant value of $p < 0.05$, which means there is a correlation, shows a value of $I = -0.132$, which means that the relationship between the percentage of mosquito deaths and the number of dengue fever cases is inversely proportional. The results of the study obtained the equation of the linear regression line is $Y = 174.201 - 1.21332 X$

Where Y = number of dengue cases and X = percentage of mosquito deaths

Discussions

The results of exposure to organophosphate insecticides of the malathion type in *Aedes aegypti* mosquitoes in this study gave results from 11 (eleven) Districts, there was 1 (one) District of *Aedes aegypti* mosquito mortality less than 90%, namely Pare District, while ten (10) *Aedes* mosquito mortality districts *aegypti* above 90% at 60 minutes. Research conducted by Endang tested the toxicity of organophosphate

and carbamate insecticides on *Aedes aegypti* mosquitoes, from the two selected insecticides, chlorpyrifos type organophosphate and methyl carbamate type, he concluded that these two materials were still less effective in vector control compared to organophosphate insecticides. malathion (Endang Puji Astuti, 2010). The same result was carried out by Hasyimi who examined four insecticides, namely Malathion, Fendona, Icon and Cynoff. The four insecticides that were most effective in reducing larval numbers were malathion, while icon and cynoff could reduce adult mosquito populations but could not reduce larvae numbers. (M Hasyimi, 2006).

Malathion is an organophosphate insecticide, which works by binding to the acetylcholinesterase enzyme found in mosquitoes. This enzyme is mosquito detoxification located in the central nervous system, serves to break down enzymes into acetic acid and choline, if these enzymes are bound, the enzymes do not work according to their functions, as a result, acetylcholine accumulates because no enzyme breaks down and eventually the mosquito will die. The increase in the acetylcholinesterase enzyme was proven by Ming with the results of Gunandini and Wicaksana's research which described the *Aedes Aegyptus* mosquito with malathion, with an increase in the value of acetylcholinesterase activity above 30%, it was declared resistant, this resistant process occurs when an increase in the activity and function of the acetylcholinesterase enzyme can still break down acetylcholine into acetic acid and choline, despite continuous malathion (Ming An Shi, 2004). This shows that the nervous system is still running normally (DJ Gunandini, 2005).

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Distribution of Conventional Resistance of *Aedes aegypti* Mosquitoes exposed to 5% Malathion Insecticide.

Based on the criteria set by WHO, there are three criteria to determine the status of mosquito resistance to insecticides. according to (Velayudhan, 2016) The status of insecticide resistance (insecticide susceptibility) is divided into three criteria, namely 98% of insect mortality is considered susceptible, if insect mortality ranges from 90-97%, it is declared tolerant and if insect mortality < 90% is declared resistant.

The results obtained from eleven sub-districts are only one sub-district, namely the Pare area of *Aedes aegypti* mosquitoes which are resistant to 5% malathion, while ten sub-districts of *Aedes aegypti* mosquitoes are declared susceptible / Anugrah who examined resistance to malathion in *Aedes Aegyptus* mosquitoes in dengue-endemic areas of Makassar City, gave the same results, some areas of Kaluku Bodoa were still tolerant to malathion 0.8%, while some areas of Kaluku Bodoa and Kapasa showed 100% of mosquitoes experienced mortality which resulted in sensitivity/susceptibility to 6% malathion. This shows that the malathion insecticide used in the ten sub-districts can still be used to control the *Aedes aegypti* mosquito (Anugrah, 2018).

Sucipto in 2011 stated that resistance is a process of the ability to live things, namely insects, to survive in certain doses of insecticide chemicals, which in general can kill the species. This resistance can occur when a living species is exposed to it continuously (Sucipto, 2011). The susceptibility test method for the insecticide malathion 0.8% and cypermethrin 0.05% in the *Aedes aegypti* mosquito population showed resistance to both insecticides. (Miko Sudiharto, 2020). The same results showed that the *Aedes aegypti* mosquito in the Pekalongan area of Central Java also showed resistance to malathion, by knowing

the percentage of mosquito mortality that was aligned with the increase in the esterase enzyme. (Widiastuti & Ikawati, 2016).

The distribution of cypermethrin resistance in *Aedes aegypti* mosquitoes conducted by Sayono showed the spread of *Aedes aegypti* resistance to pyrethroid insecticides in the city and an increase in mosquito population density that exceeded the standard set by WHO with HI by 5%. The mosquito resistance is the result of the frequency of fogging which is carried out 2 times a year which is officially carried out by Health centre officers, but fogging is also carried out by non-governmental organizations. As a result, the frequency of fogging is difficult to determine (Sayono, 2012). Uncontrolled application of thermal fogging results in resistance to mosquitoes. The results of the study led the Indonesian government to make the right steps in the use of spraying applications in each area, that the use of thermal fogging can be done when there is a case of dengue fever and carried out 2 cycles. (Permenkes, 2017).

The local government of Kediri Regency continues to use organophosphate insecticides of the malathion type and the use of these insecticides has been carried out for a long period of more than ten years, usually, the use of organophosphate insecticides of the malathion type which is used for a long time will cause resistance to these living things.

The results of this study indicate that the spraying carried out in Kediri Regency in eleven sub-districts is not carried out continuously, so this has an impact on the *Aedes aegypti* mosquito, which shows that most mosquitoes in that location are not immune to the organophosphate group insecticide malathion 5%. So that the malathion insecticide can still be used by the local government as a control for the *Aedes aegypti* mosquito, it's just that the thermal fogging rule must be applied, which is done in 2 cycles. Because for now, the status in Kediri Regency is still in a vulnerable status, but over time and cases of dengue fever remain high, it is likely that the mortality status of mosquitoes will turn resistant. Mosquito resistance can be avoided until the F5 offspring, for the next offspring it is possible to change. This is evidenced by Isfanda that the method used is single induction selection which is exposed to three insecticides, namely malathion 0.8%, deltamethrin 0.025% and bendiocarb 0.1%. susceptible strain, the possibility of homozygous strain can be formed over five generations (Isfanda, 2017).

The relationship between *Aedes aegypti* mosquito resistance in Malathion and Dengue Fever Cases in 2019 in 11 (Eleven) Sub-Districts

The results obtained from the relationship between the percentage of *Aedes aegypti* mosquito mortality and the number of dengue fever cases in eleven Kediri Districts had an inverse relationship. This shows that the status of mosquitoes in Kediri Regency is almost all vulnerable status, meaning that the organophosphate group of malathion insecticides can still be used, but in Kediri Regency the number of dengue fever cases is still relatively high. Judging from the geoda map which shows the sub-districts in Kediri Regency that have the highest risk in cases of dengue fever are Pare Districts.

Mosquito susceptibility is not the main indicator for the reduction of dengue fever cases. Cases of dengue fever can be controlled by various factors. The main factor that must be controlled is the environmental factor of the house and its surroundings. This is evidenced by Wanti that the risk of DHF is not only climate, humidity and temperature, the most supportive factor is the condition of the house, namely lighting, water use, wall construction, ventilation size and clean water facilities. (Wanti, 2019).

Mosquito populations can be controlled by fogging as is done by the local government of Kediri Regency, according to an article written by Elsa measuring mosquito density in the field as an evaluation in fogging implementation in 2016, in an area with the highest dengue cases, in September in the rainy season. rain. Elsa Endiyani in her research used organophosphate insecticide zetta cypermethrin which was mixed with diesel fuel, with a ratio of 0.5 zetta cypermethrin/10 liters of diesel and carried out two (2) times fogging cycles in stages. The results obtained 2 days after fogging 1 experienced a decrease in mosquito density, but on day 3 after fogging the density of *Aedes aegypti* mosquitoes increased. The mosquito density decreased on the 3rd and 6th day after the 2nd fogging, but on the 4th day after the 2nd fogging it also increased, this shows that fogging 2 times cycles is very effective in reducing the density of the *Aedes aegypti* mosquito. This shows that the mosquito population cannot be killed, fogging only kills adult mosquitoes, while the eggs will continue to breed, if not controlled, the mosquito density will increase. (Elsa Endiyani, 2016).

More effective control of mosquito population/density by doing Drain and brush water reservoirs regularly. Close tightly all water storage areas. Utilizing used waste that has economic value (recycling) Plus and keeping the house clean.

Conclusions

The mortality percentage of *Aedes aegypti* mosquitoes exposed to Malathion 5% with exposure within 60 minutes is less than 90% which means it is resistant to malathion, namely Pare District, while 10 (ten) other districts are above 90% (vulnerable). The relationship between the percentage of deaths of *Aedes aegypti* mosquitoes exposed to the Malathion insecticide to Dengue Fever Cases in 2019 in 11 (Eleven) Districts of Kediri Regency obtained significant results with a P-value < 0.05, with the results of the correlation being inversely proportional to the value $r = -0.132$ and from the mapping it was obtained The cluster that has a high risk is the Pare sub-district

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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