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THE RESISTANCE STATUS OF *Aedes Sp* LARVAE TO *TEMEPHOS* IN THE PERIMETER AND BUFFER AREAS OF SURABAYA TANJUNG PERAK SEAPORT

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ABSTRACT
The seport was the gateway transmission of the disease. IHR 2005 stated that the perimeter and port buffer areas have to be free from larvae to maintain these conditions KKP Class 1 Surabaya conducts continuous control by employ the larvacidae with the activated ingredient *temephos*, that has been used for more than ten years and can trigger resistance. The purpose of this study to determined the resistance status from *Aedes sp* larvae to *temephos* in the Perimeter and Buffer area of the Surabaya Tanjung Perak Seaport. Type of research used experimentation purified design with Postest Only with Control Group Design. The research sample were the third generation (F3) *Aedes Sp* instar III larvae used *temephos* with a variation concentrated 0.01 mg/L; 0,02 mg/L; 0,03 mg/L; and 0,04 mg/L with 24-hour contact time. Data analysis in determined the resistance status with reference to the standard categories of WHO. The test results which was conducted a total of five times indicated test results of the status vector based on standards WHO that the status larvae against *temephos* with variations concentrated 0.01 mg/L; 0.02 mg/L; 0.03 mg/L; and 0.04 mg/L was resistant. The concluded resistance status of *Aedes Sp* larvae to *temephos* in the Perimeter and the Buffer area have indicated resistant. Suggestion for the KKP Class 1 Surabaya to conduct further research on status resistance larvae *Aedes Sp* to *temephos*.
Keywords: ***Resistance, Aedes Sp, Temephos, Larvicides, Port***

BACKGROUND
The port is a meeting place or activity in and out of ships, goods and people as well as a gateway for the spread of disease. One aspect of disease transmission at ports is through disease-transmitting insects (vectors), as well as nuisance animals, both carried from transportation and those already present at the port. Decree of the Minister of Health Number 431 concerning Control of Environmental Health Risks at Ports/Airports/Transboundary Posts in the Context of Health Quarantine, the House Index (HI) of *Aedes Sp* in the buffer area is less than 1%. Meanwhile, in the perimeter area of *Aedes Sp* mosquitoes, neither the larval stage nor the adult stage can be found in the perimeter area.

One of the controls carried out in controlling the impact of environmental health risks is control of quarantine disease vectors. IHR, 2005 stipulates yellow fever as one of the quarantine diseases transmitted by *Aedes Sp* mosquitoes infected with the virus that causes yellow fever. This disease is one of the most dangerous infectious diseases. The mortality rate of this disease ranges from 20-50%, but in severe cases it can exceed 50%.

Dengue Hemorrhagic Fever is still a health problem in Indonesia. The incidence rate (incidence rate) or dengue hemorrhagic fever (DHF) in East Java in 2021 is 17 per 100,000 population, which is in accordance with the national target incidence rate, which is 49 per 100,000 population. The DHF case fatality rate (CFR) in 2020 is 0.8%, but in 2021 it is 1.1%, this shows that the death rate due to DHF in East Java is still above the national target set, which is <1%. While the achievement of the larvae-free rate in 2020 is 89%, and in 2021 it is 90% lower than the target that has been set, which is 95%. The risk of transmission of dengue cases in East Java tends to be influenced by population density, population mobility, urbanization (East Java Provincial Health Office 2021).

Strategic steps that can be taken for prevention and control of dengue cases are community empowerment efforts and community participation in 3M Plus Mosquito Nest Eradication activities at least once a week on a regular basis. The activity of eradicating the nests of *Aedes Sp* mosquitoes at the larval stage was carried out by applying chemical-based larvicides with the active ingredient *temephos*. The use of *temephos* in Indonesia has been started since 1976. In 1980, *temephos* was established as part of the *Aedes Sp* eradication program in Indonesia. Until now (in 2021), *temephos* is still used to kill larvae of *Aedes Sp*. This means that the use of *temephos* in Indonesia has been around for 40 years (Cahyati et al. 2019). The use of larvicides for a long time can cause vector resistance to these larvicides (Natalina et al. 2019).

Resistance develops in vector species populations through generation or selection due to insecticide exposure to vector species and the application method, dose, and scope of intervention. Vector resistance to insecticides is a global phenomenon, especially the management of vector-borne disease control programs in Indonesia. Resistance is hereditary and is the single obstacle to successful chemical vector control. Determination of the status of resistance to insecticides can be useful as program information for the selection of appropriate insecticides in vector control. The slower resistance is detected, the greater the loss caused, because insecticides are no longer effective in breaking the chain of transmission, will result in inefficiency in financing efforts to control vector-borne diseases (Sunaryo et al. 2018).

The causes of resistance are the use of the same or similar insecticides in each phase of the life of the *Aedes Sp* mosquito continuously for a long period of time, the use of active ingredients or formulations that have the same activity, long residual effects and the use of the same insecticide against all stages of vector growth. If there is resistance to insecticides, it will make the insecticides can no longer be used to eradicate a vector. For example, if the mosquito larvae are already resistant to a larvicide, then the larvicide given will no longer be able to inhibit the growth of mosquitoes into adults (Cahyati et al. 2019).

The phenomenon of resistance has been reported that *Aedes Sp* larvae have been resistant to *temephos*. Study (Nur Handayani et al. 2019) in the Perimeter and Buffer areas of Tanjung Emas Port, Semarang City, which reported that *Aedes Sp* larvae from the Tanjung Emas Port perimeter area were tolerant to *temephos*, with an average mortality of 96%. Meanwhile, in the Tanjung Emas Port Buffer area, it was reported that it was resistant to *temephos* with an average mortality of 68%. Other research conducted by (Marlik et al, 2018) The results showed that *Aedes aegypti* mosquitoes in Kediri Regency were resistant

to 0.8% malathion, while the use of 5% malathion was in the tolerant category within 60 minutes. *Aedes aegypti* larvae in Kediri Regency were resistant to *temephos* with concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l, 0.04 mg/l.

The purpose of this study was to determine the resistance status of *Aedes Sp* larvae in the perimeter and buffer areas of the Surabaya Tanjung Perak Seaport.

RESEARCH METHODS

Pure experimental research with Posttest Only with Control Group Design using WHO susceptibility test method. The concentration of *temephos* used was 0.01; 0.02; 0.03 and 0.04mg/l. In this test, there were 4 (four) concentrations and controls were carried out with 5 repetitions. The sample size according to WHO standards for resistance testing is 25 *Aedes Sp* larvae for each treatment and repetition. The total number of samples was 1250 larvae of *Aedes Sp*.

The selection of the location for taking *Aedes Sp* larvae to get the first generation was done by simple random sampling, namely 100 houses by taking a radius of 100-200 meters from the patient's house according to the farthest flight distance of *Aedes Sp* mosquitoes in the buffer area and 5 places inside the port fence in the area. perimeter.

Sampling was done by setting an egg trap (ovitrap) at the respondent's house for 7 days. Ovitrap is made dark in color and placed in a dark place according to the nature of the female mosquito at rest. Ovitrap was taken after 7 days, then rearing mosquito eggs to be bred into larvae in the laboratory. The larvae tested were late third instar larvae or early fourth instar larvae and then a susceptibility test was carried out according to WHO procedures.

Materials and tools used for resistance testing, water pH measurement; water temperature; air temperature; humidity is a pipette, plastic cup, label paper, stopwatch, aquades, insecticide *temephos* solution 156.25mg/l (then the dilution is made to 0.625mg/l and the next dilution is 0.01mg/l; 0.02mg/l; 0.03mg/l and 0.04mg/l), *Aedes Sp* larvae, pH stick, thermohygrometer.

Resistance testing was carried out using the Larval Mosquito Susceptibility test method according to WHO standards. The WHO standard category resistance criteria are:

a. Death 98% = Susceptible/vulnerable/sensitive
b. Death between 90 - 97% = Tolerant
c. Death < 90% = Resistance

RESULTS

1. Conditions of room temperature, room humidity, water temperature and pH of the physical environment and water media on larval resistance testing *Aedes Sp*

Resistance testing was carried out by taking into account the control variables, namely room temperature, room humidity, temperature and pH of the water. Larvae resistance test activities *Aedes Sp* carried out on different days and each day 5 replications were carried out in the perimeter and buffer areas of the Tanjung Perak Seaport. The results of measurements of room temperature, room humidity, temperature and pH of the water during the test can be seen in table 1 and table 2 below.

Table 1. Results of Measurement of Room Temperature, Room Humidity, Water Temperature and pH

Origin of Test Biota Region	Room Temperature (°C)	Room Humidity (%)	Water Temperature (°C)	Water pH
Perimeter Area				
Replication I	32	80	28	7
Replication II	32	80	28	7
Replication III	34	75	29	7
Replication IV	32	80	29	7
Replication V	34	75	28	7
Buffer Region				
Replication I	34	75	29	7
Replication II	34	75	28	7
Replication III	33	80	28	7
IV Replication	33	80	29	7
V . replication	33	80	28	7
Average	**33.1**	**78**	**28.4**	**7**

Based on the table above, it can be seen that the air temperature in the study room ranged from 32-34°C and the average room temperature was 33.1°C. Air humidity in the study room ranges from 75-80% and the average humidity in the room is 78%. The water temperature ranges from 28-29°C and the average water temperature is 28.4°C. The average pH of the water used by the larvae is 7.

2. Larvae mortality percentage *Aedes Sp* in the control group and the treatment group against *temephos* with concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l, and 0.04 mg/l exposed within 24 hours

The number of larval deaths in the control and treatment groups is presented in tabular form. Number of deaths in control and treatment of *temephos* with concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l, and 0.04 mg/l in larvae *Aedes Sp* with a contact time of 24 hours with 5 (five) repetitions in the perimeter and buffer areas can be seen in table 2.1 and table 2.2.

Table 2.1 Larvae resistance test results *Aedes Sp* in the control groupwith a contact time of 24 hours

Replication	Number of Larva	Perimeter Area			Number of Larva	Buffer Region		
		f	Average	%		f	Average	%
I	25	0	0.0	0.0	25	0	0.0	0.0

II	25	0	0.0	0.0	25	0	0.0	0.0
III	25	0	0.0	0.0	25	0	0.0	0.0
IV	25	0	0.0	0.0	25	0	0.0	0.0
V	25	0	0.0	0.0	25	0	0.0	0.0
Total	125	0	0.0	0.0	125	0	0.0	0.0

Based on the table above, it is known that in the perimeter and buffer areas the number of deaths of *Aedes Sp* larvae in the control group exposed for 24 hours on average was 0 with a percentage of 0%. During the 24-hour contact time, no larvae died in replication I to V replication for the control group.

Table 2.2 Larvae resistance test results *Aedes Sp* in the treatment group against *temephos* with a contact time of 24 hours

Replication	Concentration (mg/l)	Number of Larva	Perimeter Area			Number of Larva	Buffer Region		
			f	Average	%		f	Average	%
I	0.01	25	0	0.0	0.0	25	0	0.0	0.0
	0.02	25	0	0.0	0.0	25	0	0.0	0.0
	0.02	25	0	0.0	0.0	25	0	0.0	0.0
	0.04	25	0	0.0	0.0	25	0	0.0	0.0
II	0.01	25	0	0.0	0.0	25	0	0.0	0.0
	0.02	25	0	0.0	0.0	25	0	0.0	0.0
	0.02	25	0	0.0	0.0	25	0	0.0	0.0
	0.04	25	0	0.0	0.0	25	0	0.0	0.0
III	0.01	25	0	0.0	0.0	25	0	0.0	0.0
	0.02	25	0	0.0	0.0	25	0	0.0	0.0
	0.02	25	0	0.0	0.0	25	0	0.0	0.0
	0.04	25	0	0.0	0.0	25	0	0.0	0.0
IV	0.01	25	0	0.0	0.0	25	0	0.0	0.0
	0.02	25	0	0.0	0.0	25	0	0.0	0.0
	0.02	25	0	0.0	0.0	25	0	0.0	0.0
	0.04	25	0	0.0	0.0	25	0	0.0	0.0

V	0.01	25	0	0.0	0.0	25	0	0.0	0.0
	0.02	25	0	0.0	0.0	25	0	0.0	0.0
	0.02	25	0	0.0	0.0	25	0	0.0	0.0
	0.04	25	0	0.0	0.0	25	0	0.0	0.0
Total		500	0	0.0	0.0	500	0	0.0	0.0

Based on the table above, it is known that in the perimeter and buffer areas the number of *Aedes Sp* larvae mortality from all *temephos* concentrations exposed for 24 hours on average is 0 with a percentage of 0%. During the 24-hour contact time, no larvae died in replication I to V replication for groups exposed to concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l and 0.04 mg/l.

3. Determination of resistance status of *Aedes Sp* larvae in the perimeter and buffer area of Tanjung Perak Port Surabaya after a contact time of 24 hours

Determination of resistance status can be done after counting the number of larvae that died to the concentration *temephos*. Determination of resistance status based on the calculation of the number of larvae that were in contact at concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l and 0.04 mg/l after 60 minutes of observation divided by the total larvae and made into the form of percent. Then the results are set according to the WHO category (2016). Based on the percentage results for determining larval resistance status *Aedes Sp* can be seen in table 3 below.

Table 3. Larvae resistance status *Aedes Sp* to *temephos* in the perimeter and buffer area of the Surabaya Tanjung Perak Seaport

Region	*Temephos* Concentration (mg/l)			
	0.01	**0.02**	**0.03**	**0.04**
Perimeter	Resistance	Resistance	Resistance	Resistance
buffer	Resistance	Resistance	Resistance	Resistance

Based on the table above, it can be concluded that the resistance status of *Aedes Sp* larvae originating from the Perimeter and Buffer regions to *temephos* is resistant according to the WHO standard category (2016). *Aedes Sp* larvae from Perimeter and Buffer regions were all resistant to *temephos* with concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l and 0.04 mg/l.

DISCUSSION

1. Conditions of room temperature, room humidity, water temperature and pH of the physical environment and water media in testing *Aedes Sp* larvae resistance

a. Room temperature

Temperature is a control variable that can affect research results, it needs to be controlled by measuring and analyzing. Temperature measurements were carried out during the study, namely 5 replications for 2 different areas. The measurement

results during the study were 32-34°C and the average room temperature of the study was 33.1°C.

Air temperature plays an important role in the evaporation of water and also the ability to hold water in the air and chemical processes in the air. The higher the air temperature, the higher the evaporation rate of water, the higher the water vapor. The lower the air temperature, the ability to hold water vapor also decreases. The temperature in the research room can affect the water temperature in the research room. The water temperature can adjust to the environment around it, if the temperature in the room is low, the water temperature is also low and vice versa. In this case, the room temperature needs to be controlled so as not to affect the temperature of the water used by *Aedes Sp* larvae during the test.

b. Room Humidity

Air humidity is a control variable that can affect research results, it needs to be controlled by measuring and analyzing. Measurement of air humidity was carried out during the study, namely 5 times replication for 2 different areas. The measurement results during the study were 75–80% and the average humidity of the research room was 78%. The humidity in the research room is in optimal condition and does not affect the results of the study. This is supported by (WHO, 2009) that 45% humidity is the lowest limit for mosquito life. At 70% humidity mosquitoes can survive approximately 100 days.

At low air humidity will cause evaporation of water in the body of *Aedes Sp* which will result in dryness of mosquito body fluids. Therefore, one of the enemies of adult mosquitoes is evaporation. The optimal average humidity for the development of *Aedes Sp* mosquito larvae ranges from 70-90% (Pramurditya et al. 2017).

The low survival of *Aedes Sp* is caused by a slow metabolic process due to low temperature and humidity so that it can lead to the death of larvae. Based on this we know that humidity that does not meet the requirements will result in the death of the larvae, thereby reducing the chances of finding larvae. One of the factors that may affect the results of this humidity measurement is due to the time of the study which coincides with the rainy season so that the water vapor content in the air increases (Sulfiani et al. 2021).

c. Water Temperature

Water temperature is one of the factors that can affect the survival of *Aedes Sp*. larvae. Water temperature plays a role as a determinant of the success of larval growth. The results of the research on water temperature in the research room were replicated 5 times for 2 different areas. The measurement results during the study were 28–29°C and the average water temperature used by the larvae was 28.4°C. The water temperature used by the larvae during the resistance test was in accordance with the recommended range with the optimal water temperature for larval life. This is also supported by research (Ridha et al. 2011), stated that water temperature was related to the presence of *Aedes Sp*. larvae. In this study, the water temperature that supports the presence of *Aedes Sp* larvae is 25-30°C. This means that the temperature is ideal for larval development and growth. The growth of *Aedes*

Sp larvae will be disturbed if the water temperature is less than 25°C or more than 30°C.

Water temperature is one of the factors that can affect the development and survival of *Aedes Sp* larvae, the appropriate water temperature for the development of *Aedes Sp* larvae (Wahyuni, 2013). One of the environmental parameters that is significantly related to the population density of *Aedes Sp* mosquito larvae is water temperature. Water temperature plays a role as a determinant for the success of larval growth. The cause of the uniformity of the water temperature in the container is due to the relatively low environmental temperature at the time of the study because the weather is always cloudy and tends to rain (Sulfiani et al. 2021).

d. Acid Base (pH) of Water

The degree of acid base (pH) of water is a factor that greatly determines the growth or survival of *Aedes Sp*. larvae. Water pH is a control variable that can affect research results, it needs to be controlled by measuring and analyzing. Measurement of water pH was carried out during the study, namely 5 replications for 2 different areas. The result of measuring the pH of the water used by the larvae during the study was 7. So it can be concluded that the pH of the water used for the larvae was still in the optimum range for the larvae to grow and did not affect the performance of the active ingredient *temephos* being studied on the mortality of *Aedes Sp*. larvae. It is supported by (Yahya et al. 2019) which states that optimal larval growth occurs in the pH range of about 7 and larvae will die at pH 3 and 12.

pH is a factor that greatly affects the life of *Aedes aegypti* larvae. The pH of the water that is too acidic or too alkaline will easily lead to the death of the larvae. One of the factors that can affect the survival of larvae is the availability of food. A pH that is too acidic is thought to inhibit the growth of plankton while it is known that plankton is one of the largest food sources for larvae, with reduced food sources for larvae the chances of larvae to survive are very small (Sulfiani et al. 2021).

2. Death of *Aedes Sp* larvae in the control group and treatment group to *temephos* with concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l, and 0.04 mg/l exposed within 24 hours

Larvae of *Aedes Sp* from Perimeter and Buffer area of Tanjung Perak Seaport Surabaya exposed using *temephos* at concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l and 0.04 mg/l for 60 minutes and after 24 hours no *Aedes Sp* larvae died. The results of the calculation of the percentage mortality of *Aedes Sp* larvae against the control group and the treatment group were 0% in the Perimeter and Buffer areas of the Tanjung Perak Seaport Surabaya.

Almost all *Aedes Sp* mosquito larvae in the Perimeter and Buffer areas at varying concentrations did not die, this was exacerbated by the fact that *temephos* had been used for almost 40 years (since 1976) in Indonesian society in controlling *Aedes Sp*. In its use, *temephos* often does not reach the effective concentration recommended by the Indonesian Ministry of Health. Larval resistance can be hereditary which is transmitted through the parent. The mechanism of insect resistance to insecticides consists of 3 ways, namely increasing the detoxification (become non-toxic) of insecticides due to the work of certain enzymes (Ramayanti & Febriani 2016).

Larvicide or *temephos* is an organophosphate group insecticide that has the ability as a poison that affects the neurotransmitter system. Based on three mechanisms of resistance to an insecticide, it is possible that *temephos* has detoxified the microsomal oxidase, glutathione transferase, hydrolase and esterase enzymes as well as decreased sensitivity of the insecticidal target site on the mosquito's body, in this case *acetylcholinesterse*. The decrease in the rate of penetration of insecticides through the skin is caused by the occurrence of tolerance related to genetic and bioecological factors (Ipa et al. 2017)

Control of *Aedes Sp* by looking at environmental sanitation conditions that aim to reduce larval habitat is a key strategy for this vector control program. Moreover, the use of insecticides as larvicides by the community is the most common way to control *Aedes Sp*. One of the larvicides used in Indonesia is from the organophosphate group with the active ingredient *temephos.*

Temephos is a non-systemic organophosphorus insecticide. The use of *temephos* to kill *Aedes Sp* larvae has obtained approval from the World Health Organization (WHO) and the Ministry of Health and has recommended powders with the active ingredient *temephos* to be spread in puddles that have the potential as breeding places for mosquitoes. *Temephos* is a pesticide that contains a slightly toxic product (toxicity class III) so it can be used in general. The way temephos works on larvae is to inhibit the performance of enzymes in the formation of nerve cells from the insect larvae, with these conditions and abilities, mosquito larvae cannot develop and even die immediately after ingestion or contact with *temephos*.

The use of *temephos* began since the government launched the abatization program in 1980 to break the chain of transmission of dengue fever(Ministry of Health RI 2012).This program is carried out continuously throughout the year without any larvicide rotation with the aim of avoiding outbreaks. According to Georgio, insect resistance to an insecticide will occur if it is used intensively for 2 to 20 years and continuously throughout the year (Georghio et al. 1988).

Resistance is the ability of a vector population to survive against a dose of insecticide that under normal circumstances can kill the vector species. To determine the status of resistance or susceptibility of insecticides to insects, it is necessary to conduct a resistance test. The resistance test will produce data on the number of larvae mortality after being exposed to insecticides for 24 hours which is then determined according to the standard resistance status.

The resistance test was carried out in the negative control group which was tested using distilled water and the treatment group using the organophosphate insecticide, namely *temephos* with variations in concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l and 0.04 mg/l. In the negative control group, which was tested for 24 hours using distilled water, there was no death of *Aedes Sp* larvae, so there was no need to make corrections with Abbot's formula. In the treatment group using *temephos* with concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l and 0.04 mg/l exposed for 24 hours did not experience death. The *Aedes Sp* larvae resistance test was replicated 5 times to obtain valid data for each control group and treatment group.

Aedes Sp larvae in the Perimeter and Buffer regions at various concentrations did not die. The reason is that the concentration used is too small so it is not able to work to kill *Aedes Sp* larvae. Insecticides that exist and are used by the community to date are generally *temephos*. The dose rule for *temephos* in the national larvicidation program in the community is 10 grams/100 liters or 100 mg/l, so the dose used by the community is 1%. Meanwhile, in this study, abate material was used, namely *temephos* with concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l and 0.04 mg/l and no larvae died at these concentrations. This is because the toxicity of *temephos* used on larvae is too small.

Other research conducted (Marlik et al. 2018) on Conventional Detection of *Aedes aegypti* Resistance as DHF Vector in Kediri Regency against *Malathion* and *Temephos* using *temephos* concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l and 0.04 mg/l. The result was that there was no death of *Aedes aegypti* larvae, so it can be said that *Aedes aegypti* larvae in Kediri Regency were resistant to *temephos* with concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l and 0.04 mg/l.

3. Resistance status of *Aedes Sp* larvae to *temephos* in the perimeter and buffer areas of the Surabaya Tanjung Perak Seaport

Resistance is an individual insect's ability to survive against a dose of insecticide that under normal circumstances can kill the insect species. Insecticide resistance status against insects was measured using a resistance test procedure, which is an appropriate standard method for measuring insecticide resistance, especially in the field (Marlik et al. 2018). The standard test for *Aedes Sp* larvae resistance was obtained in the test with the following percentage of mortality; larvae are categorized as vulnerable if the mortality of the test larvae is 98-100%; categorized as tolerant if the mortality of the test larvae was 90-97% and categorized as resistant if the mortality of the test larvae was 90%.

Based on the results of the study, *Aedes Sp* larvae in the Perimeter and Buffer areas of Tanjung Perak Seaport Surabaya were categorized as resistant to *temephos* with concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l and 0.04 mg/l. l. This was proven based on the results of resistance tests from two areas at the Tanjung Perak Seaport, Surabaya, which had a mortality percentage of test larvae below 90% during 60 minutes of observation and after 24 hours at concentrations of 0.01 mg/l, 0.02 mg/l, 0. 03 mg/l and 0.04 mg/l.

The recommended follow-up if resistance to insecticides has occurred, alternative control is to rotate using insecticides that work differently and ideally use a two-year cycle; intervene on larvae and adult mosquitoes simultaneously with insecticides that have different ways of working, for example adult mosquitoes use the organophosphate group and larvae use the Insect Growth Regulator (IGR); and using insecticides that have different working methods that are applied based on geographical areas, for example, region A uses the pyrethroid group and region B uses the organophosphate group.

Another alternative solution is to use new insecticides to eradicate these insects. This is because, if the dose is continuously increased without a new insecticide replacement

plan, it will one day endanger public health and environmental health. If an effective dose in the future cannot kill *Aedes Sp* larvae effectively and it is proven that disease transmission continues to occur, then *temephos* (abate) must be immediately replaced with other active ingredients that have a different way of working with *temephos* (abate). Insecticide rotation also needs to be carried out for vector control with a maximum period of 2-3 years or 4-6 times of application according to (Ministry of Health RI 2012). So it is necessary to do further research on doses that can endanger the health of living things, especially humans, if the use of *temephos* dose is increased.

With regard to replacing the active ingredient of larvicides, it is necessary to pay attention to the physiological system of the insecticide targets previously used. It is better when resistance occurs, the change of active ingredients should use materials whose physiological targets of insecticides are different from before. The physiological system that becomes the target point of insecticides that interfere with the function of the neurotransmitter nervous system. *Temephos* larvicide derived from the organophosphate group is an insecticide that works as an *acetylcholine* (AChE) inhibitor.

According to WHO recommendations, other alternatives to replace insecticides or rotate their use other than *temephos* can switch to using Insect Growth Regulators (IGR) such as *pryproxyfen, altosid, metoprene,diflubenzuron*, etc. In contrast to the organophosphate group which targets the nervous system, IGR targets the development and growth system. IGR is a chemical that can interfere with and inhibit the growth hormone of larvae from the I to IV instars so that they do not succeed in becoming pupae or adult mosquitoes. IGR does not directly react to poison the larvae. His death was due to developmental delays since the larval stage and his inability to metamorphose.

The results of the determination of resistance that have been carried out by the authors are only based on resistance tests with 5 replications using the susceptibility test and then categorized according to WHO standards. The results of the study showing the resistance status are temporary results. This is because determining the resistance status of *Aedes Sp* larvae to *temephos* in an area requires repeated testing and further research.

CONCLUSION AND RECOMMENDATION

Conclusion

1. The research room temperature ranged from 27 - 30°C and the average room temperature was 28.1°C. The humidity in the research room ranged from 50–65% and the average humidity in the room was 59.6%. The water temperature used by the larvae ranged from 28 – 29°C and the average water temperature was 28.4°C. The pH of the water used by the larvae is 7.
2. The number of deaths of *Aedes Sp* larvae in control and treatment with concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l and 0.04 mg/l in the Perimeter and Buffer regions with 5 (five) replications times the average is 0.
3. Percentage of mortality of *Aedes Sp* larvae in control and treatment with concentrations of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l and 0.04 mg/l in the Perimeter and Buffer regions with 5 (five) replications times by 0%.
4. The resistance status of *Aedes Sp* larvae in the Perimeter and Buffer regions was temporarily resistant.

Recommendation

1. For Health Agencies
   a. Monitoring and monitoring vector resistance on a regular basis in the Perimeter and Buffer areas to determine the status of larval resistance which aims to provide input and follow-up in the mosquito vector eradication program *Aedes Sp*.
   b. Another alternative is to use insecticides from other groups that are not included in the organophosphate group, such as the pyrethroid group and to rotate the insecticide with a maximum period of 2-3 years or 4-6 times of application.
   c. Another alternative is to use*Insect Growth Regulator(IGR)*e.g. pryproxyfen, altoside, metoprene, etc.
2. For Other Researchers
   a. Further research is needed using biochemical and molecular tests to determine the mechanism of resistance that plays a role.
   b. Further research is needed on larval resistance testing *Aedes Sp* to *temephos* with concentrations above 0.04 mg/l.
   c. Further research is needed on larval resistance testing *Aedes Sp* using insecticide active ingredients other than the organophosphate group.
3. For Society

   Chemical control using insecticides, especially with the use of *temephos* must continue to pay attention to the principles of control such as the use of doses according to the recommendations of the ministry of health so as not to cause resistance. (Abate sowing can be repeated every 2-3 months. Abate use: 10 grams for 100 liters).

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