

*Regional Case Study***Differences in Before and After Temephos Intervention in Bengkulu City: Larvae Free Rate of *Aedes spp.*****Lala Foresta Valentine Gunasari¹, Rizqita Destilyana², Nikki Aldi Massardi³, Dessy Triana^{1*}**¹Department of Parasitology, Faculty of Medicine and Health Science, Universitas Bengkulu, Bengkulu, Indonesia²Medical Program, Faculty of Medicine and Health Science, Universitas Bengkulu, Bengkulu, Indonesia³Department of Anatomical Pathology, Faculty of Medicine and Health Science, Universitas Bengkulu, Bengkulu, Indonesia*Corresponding Author, email: dessy.triana@unib.ac.id**Abstract**

One of the strategies to reduce dengue transmission is to control the population of vectors, such as larval eradication. The use of temephos larvicide (Abate®) is the most widely carried out in the community. An indicator of the success of the larval eradication program is the Larvae Free Rate (LFR) assessment. This study aimed to determine the difference between LFR of *Aedes spp.* before and after temephos intervention in Bengkulu City. This study is experimental research with one group pre-test and post-test design. The population of this study was all houses in Bengkulu City with a total sample of 670 houses. Sampling was carried out using cluster random sampling techniques in 67 urban villages in Bengkulu City. This research was carried out by conducting a survey of larvae before and after temephos intervention in containers. Sixty-seven urban-villages, in 44 (65,67%) urban-villages was found an increase of LFR after temephos intervention. The average LFR before temephos intervention was 67.61%, while after temephos intervention increased to 82.98%, there was a significant difference between LFR of *Aedes spp.* before and after temephos intervention in Bengkulu City ($p=0.001$). Temephos intervention is effective to increase LFR of *Aedes spp.* in Bengkulu City.

Keywords: Dengue; temephos; insecticides; larvae free rate**1. Introduction**

Dengue Hemorrhagic Fever (DHF) is a disease caused by *Dengue* virus infection which is divided into four serotypes, which are DENV-1, DENV-2, DENV-3, and DENV-4. Transmission of the virus through the bite of the *Aedes spp.* mosquito which are *Aedes aegypti* (*Ae. aegypti*) and *Aedes albopictus* (*Ae. albopictus*) as the most dominant vectors (Pahlevi and Kesetyaningsih, 2019). Based on WHO (2020) states that from 2015 to 2019, Dengue cases increased by 46% in the Southeast Asian region (World Health Organization, 2020). Meanwhile, the mortality rate decreased by 2%. Indonesia is one of the most endemic countries in the world so that it has considerable involvement in the increase in Dengue cases (World Health Organization, 2021). Data from the Bengkulu City Health Office (2022) showed that Dengue cases reached 210 cases with an incidence rate of 34,8 per 100.000 population, while the Dengue case fatality rate (CFR) in Bengkulu City in 2022 was <1% (Bengkulu City Health Department, 2022).

One of the factors that play a role in the increase in Dengue morbidity and mortality is the proximity between the vector breeding environment and human habitation (World Health Organization, 2011). Capabilities of eggs and larvae to survive in water containers even in unfavorable conditions making it difficult to control the vector population (Ritchie, 2014). Therefore, larval eradication was chosen to be one of the main strategies of vector control programs worldwide (Hanafiah et al., 2019). Female mosquitoes *Aedes aegypti* and *Aedes albopictus* have anthropophilic characteristics and like to lay their eggs in clean water without chemical contamination, organic materials, and do not come into direct contact with the ground, such as flower vases, bathtubs, jars, and more (Agustin, Tarwotjo and Rahadian, 2017). Based on the Regulation of the Indonesian Minister of Health Number 50 of 2017, vector control can be carried out through physical, biological, chemical, and environmental management methods (Kementerian Kesehatan RI, 2017). The use of larvicide as a chemical method is popular and best used to control vectors that are at high risk of causing outbreaks (World Health Organization, 2011).

Larvicide is commonly used in the community because it is able to control all types of larvae with a mortality percentage of 95-100% (Raharjo, 2009; Kurniawan, Nurjana and Srikandi, 2019). Only a few insecticide ingredients are approved by toxicologists for use in drinking water, which are temephos, permethrin, methoprene, and products based on *B. thuringiensis israelensis* (Becker et al., 2010). The use of larvicide temephos (Abate®) in Indonesia has been done for more than 30 years (Pambudi et al., 2018). Temephos is indispensable for Dengue control because it is cost-effective, relatively safe, and the only method that provides long-term effects against Dengue vectors (Satriawan, Sindjaja and Richardo, 2019). However, the level of susceptibility of such vectors should be monitored regularly to ensure effective use of insecticides (World Health Organization, 2011). Currently, the use of temephos (Abate®) in Bengkulu City is only used during extraordinary events. According to the report of Indonesian Ministry of Health (2020b), outbreak of Dengue infection has occurred annually during the period 2016-2020 (Kementerian Kesehatan Republik Indonesia, 2020). Therefore, it is necessary to monitor and observe the influence of temephos towards larvae-free numbers in Bengkulu City as a strategy to strengthen vector management to achieve countermeasures targets Dengue by 2030 (World Health Organization, 2020).

The success of the mosquito nets eradication program in the form of larvae eradication can be estimated by calculating the Larvae Free Rate (LFR). Based on Environmental Health Quality Standards, in order to reduce the risk of Dengue transmission, the minimum LFR in an area is 95% (Kementerian Kesehatan RI, 2017). According to Indonesia's Health Profile in 2018, national LFR until 2018 has not reached the target of $\geq 95\%$ (Ministry of Health of Indonesia, 2019). In 2022, LFR in Bengkulu City has also not reached the national target since it was 85,16% (Bengkulu City Health Department, 2022). Thus, this study is intended to be able to find out the difference between LFR *Aedes* spp. before and after temephos intervention in Bengkulu City. Based on research by Rosmini et al. (2006) stated that in the Palu City area the use of temephos can increase LFR and reduce the risk of dengue fever (Rosmini et al., 2006).

2. Methods

This type of research is experimental research with one group pre-test and post-test design. Sampling was carried out in Bengkulu City with a total of 67 urban villages. The population in this study was all houses in Bengkulu City with the number of samples taken as many as 670 houses. The research location was conducted in all sub-districts in Bengkulu City (50 houses per sub-district). The sample selection in each sub-district was carried out randomly with a minimum distance between houses of 100 meters as the flight distance of the *aedes aegypti* mosquito. Sampling is done by cluster random sampling technique. The selection of samples in each urban village was carried out randomly with a distance between houses of at least 100 meters. The independent variable in this study was the intervention of temephos larvicide while the

dependent variable was the LFR in Bengkulu City. The procedures of this study are survey of larvae before intervention of temephos, temephos intervention, and survey of larvae after intervention of temephos. The larvae survey was carried out visually by observing the containers inside and outside the house. An image of a larva-free container with a container containing larvae can be seen in Figure 1. The researcher counted the number of larvae-free houses, then compared them to the overall number of houses inspected which we called LFR. The value is recorded for each urban village. furthermore, the researcher explained about larvicide to the respondent and asked permission to apply it in each container, both positive and negative larvae. The larvicide used is 1% temephos (5 grams for every 100 liters of water) packaged using zip lock plastic to facilitate dose measurement. Containers that have been treated with temephos powder should not be drained for five days. The first survey was carried out five days to ensure that the temephos given in the container was still good, data collection on larva-free numbers was carried out one month later. After five days, researchers re-observed the containers that have been treated with temephos and then recounted LFR after larvicide intervention one month later.



Figure 1. The temephos used in this research

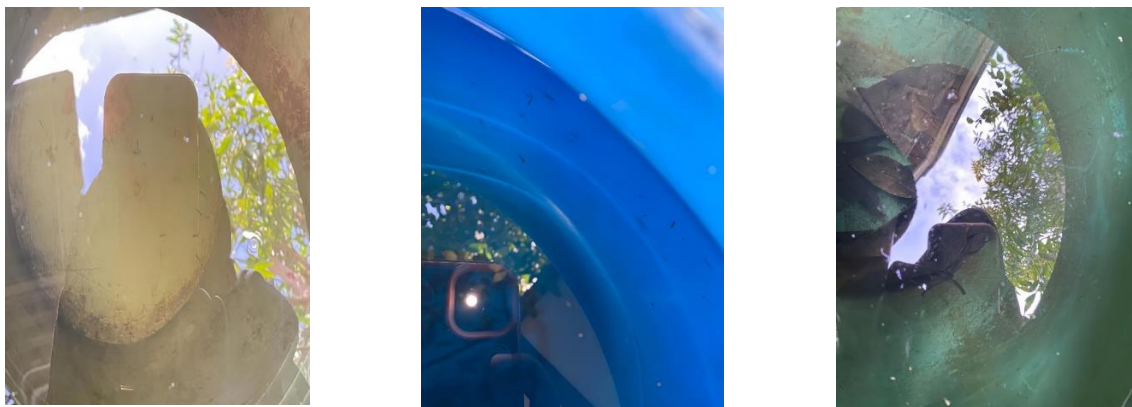


Figure 2. The containers surveyed were larvae-free and contained larvae

3. Result

3.1. Description of the Research Area

Description of the research area is an overview of the area used as a research location. Based on data from the Central Statistics Agency, Bengkulu City has an area of 151,70 km (World Health Organization, 2020). The city of Bengkulu is located at coordinates 30°45' – 30°59' South Latitude and 102°14' – 102°22' East

Longitude. To the North and East, Bengkulu City borders Central Bengkulu Regency. To the south, Bengkulu City is bordered by Seluma Regency. While in the west it borders the Indian Ocean. Bengkulu City consists of 9 sub-districts and 67 urban villages. The population in Bengkulu City in 2022 is 384,8 thousand people with 2,5 people/km² to population density (Central Data of Statistics, 2023). In this study, sample selection was carried out based on each urban village in Bengkulu City. Each urban village will take at least 7 house samples according to the calculation of the number of samples with a distance between houses of approximately 100 meters.

3.2. Larvae Survey Results Before and After Temephos Intervention

In this study, sample of 670 houses from 67 urban villages in Bengkulu City was obtained. The results of the larvae survey before and after temephos intervention in Bengkulu City are in table 1 below.

Table 1. Recapitulation of LFR n before and after temephos intervention in Bengkulu City

Neighborhoods	Examination of larvae before temephos intervention			Examination of larvae after temephos intervention		
	n (-)	N	LFR I (%)	n (-)	N	LFR II (%)
Bengkulu City	450	670	67.61	556	670	82.98
Information	: n (-)	= Number of larvae-free houses				
	N	= Number of inspected houses				
	LFR I	= LFR before temephos intervention				
	LFR II	= LFR after temephos intervention				

Table 1 shows that 450 out of 670 houses inspected were larvae-free before the temephos intervention. Based on the calculation results, the LFR of Bengkulu City before the intervention of temephos was still relatively low at 67.61% with the lowest LFR of 0%. After the intervention of temephos, the number of larvae-free houses increased to 556 houses with an average LFR of 82.98%.

The lack of larval mortality after temephos administration indicates a decrease in susceptibility and potential for resistance development in Aedes spp. larvae in several sub-districts. This decrease may be influenced by a variable dose response compared to the dose recommended by WHO. as mentioned in a study in India in 2021, showed that increasing temephos concentrations were associated with increased larval mortality, while the use of low doses had only a minimal impact. In addition, the study noted that over time, mortality rates at certain concentrations decreased, indicating the possibility of resistance development. In initial exposure, metabolic detoxification enzyme activity decreased for 2-4 generations, but repeated exposure caused a significant increase in these enzymes, which contributed to resistance to the organophosphate group. Therefore, in further studies it is important to examine resistance to several larvicides to determine their effectiveness in eradicating vector populations.

3.3. Data Analysis

The difference between average of LFR before and after temephos intervention in Bengkulu City is shown in table 2 below.

Table 2. LFR paired sample T-Test results before and after temephos intervention

Variable	n	Mean	SD	p-value
LFR before temephos intervention	67	67.6119	22.96833	0.000
LFR after temephos intervention	67	82.9851	17.66823	

To see the difference in LFR before and after temephos intervention, analysis was carried out using the Paired Sample T-Test. A significance p-value of 0.000 means that there is a significant difference in average of LFR before and after temephos intervention.

4. Discussion

4.1. Difference in Larvae Free Number of *Aedes* spp. Before and After Intervention Temephos

In this study, it was found that 44 villages (65.67%) experienced an increase in LFR after temephos intervention. LFR before temephos intervention was 67.61%, while after intervention increased to 82.98%. This shows the difference in LFR *Aedes* spp. before and after temephos intervention. The difference in LFR values is strengthened by using the Paired Sample T-Test test in Table 2, statically significant differences between LFR before and after temephos intervention in Bengkulu City. This is in line with research in Bali stated that the use of temephos can increase LFR (Kementerian Kesehatan Republik Indonesia, 2019). As how temephos works which can inhibit acetylcholineesterase (AChE) performance, causing death in larvae Central Data of Statistics, 2023).

There were 18 urban villages that have not changed LFR, and 5 urban villages that have decreased LFR. This indicates the need for further monitoring of temephos use and the effective doses that can be used in the region. The lack of larval mortality after temephos intervention indicates a reduced susceptibility and the likelihood of developing resistance in *Aedes* spp. larvae in several related urban villages (World Health Organization, 2020). Maps of changes in larvae-free rate before and after temephos intervention (Figures 2 and 3).

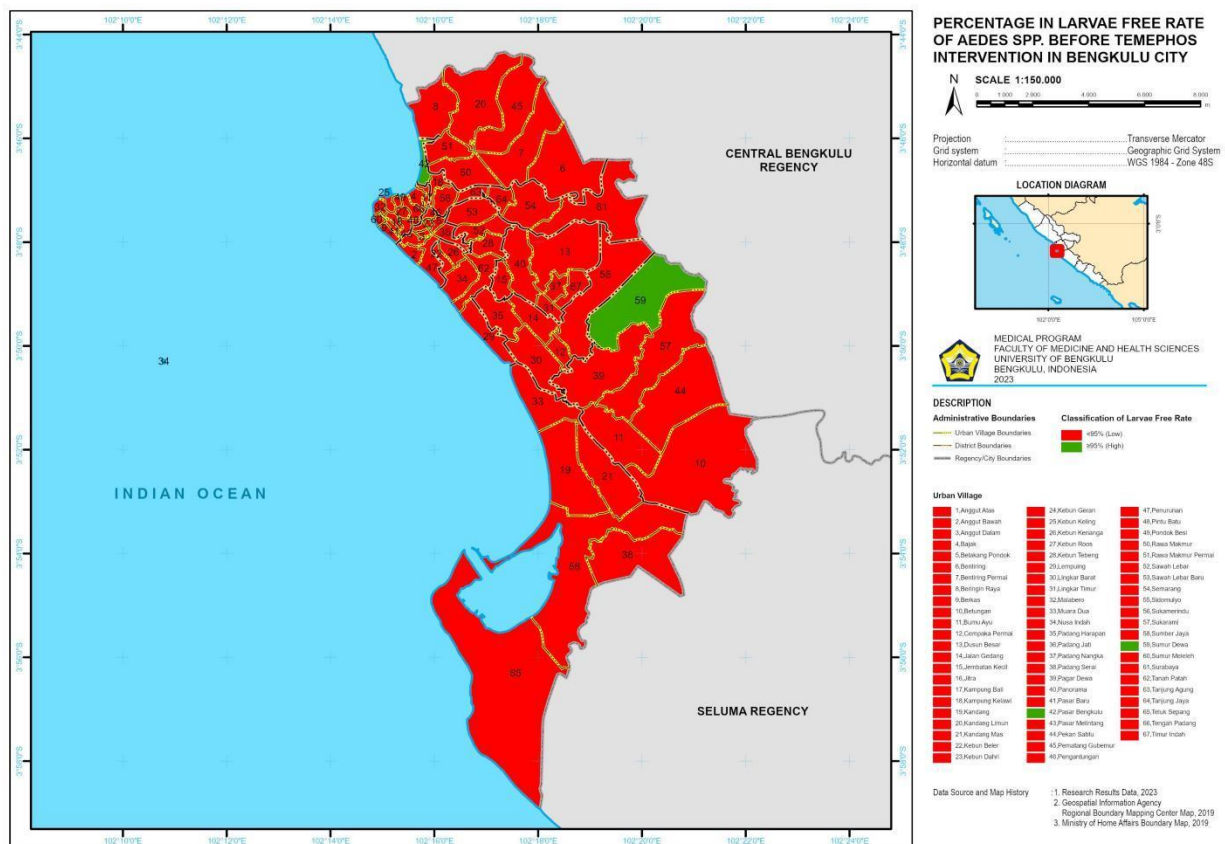


Figure 2. Larvae Free rate before temephos intervention in Bengkulu City, Indonesia

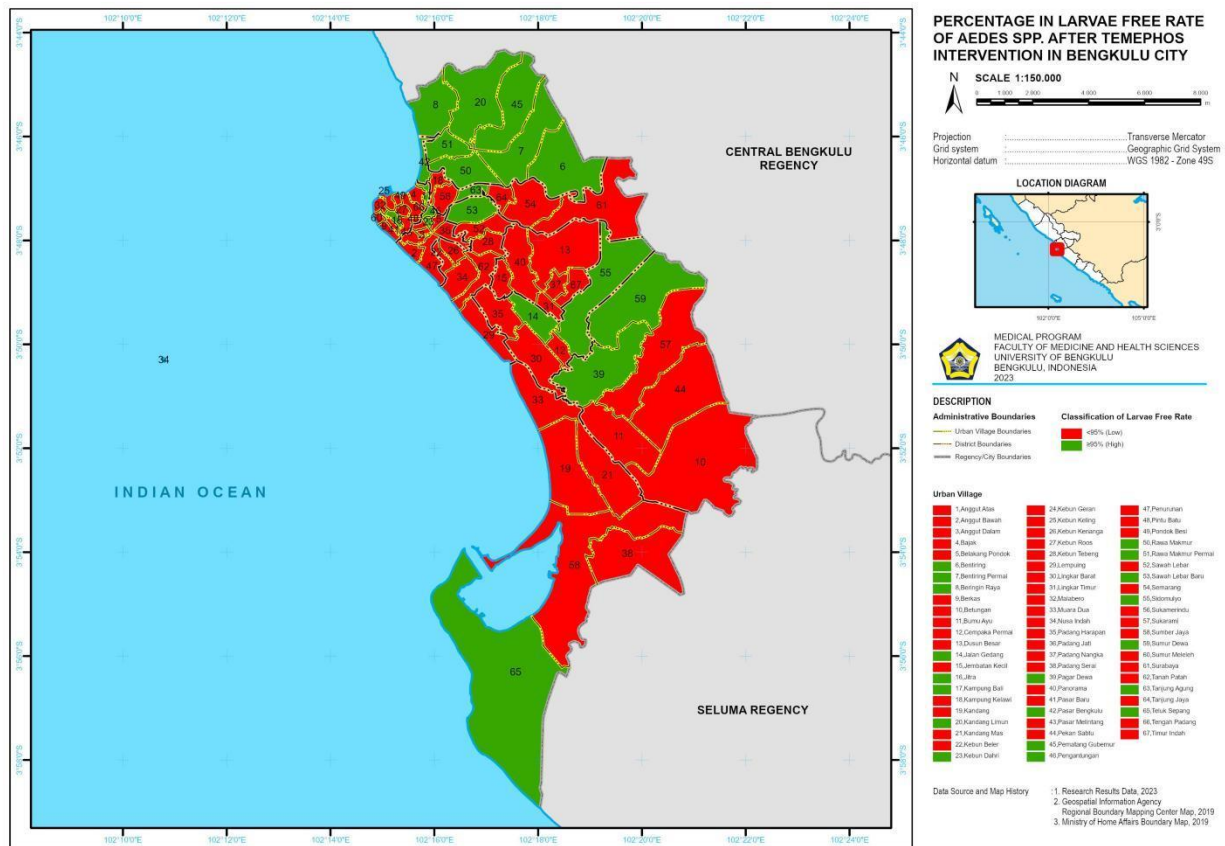


Figure 3. Larvae Free rate after temephos intervention in Bengkulu City, Indonesia

The decrease in larval mortality in some villages after temephos intervention can be influenced by dose responses that differ from the WHO recommended dose (Gan et al., 2021). One of the studies conducted in four sub-districts of Balikpapan City showed that mortality of *Aedes aegypti* against temephos had results of > 90% within 24 hours (Pratiwi and Hariani, 2020). In research conducted in India on dose response studies showed an increase in larval mortality with increasing temephos concentrations. The use of low doses has a minimal effect on the death of larvae. In addition, the study also observed that the mortality rate at certain concentrations may decrease due to the possibility of resistance. At initial exposure, the activity of metabolic detoxification enzymes decreased by 2 - 4 generations but repeated exposure caused a significant increase in these enzymes which are the enzymes responsible for organophosphate resistance (Adhikari and Khanikar, 2021).

Temephos can be absorbed by the mosquito's body by more than 99% for 24 hours (Pradani, 2020). Based on Metcalf & Luckmann (1975) in Fuadzy et al. (2015), there are several risk factors that are very influential in the occurrence of resistance, namely the type, form of formulation, dosage, method of application, frequency, and duration of time are operational factors. The frequency of insecticide use is a factor that plays a major role in the rate of development of resistance to an insecticide, especially in Dengue endemic areas. Physiological factors related to the exposure of an individual mosquito to insecticides that cause death in susceptible individuals and survive in individuals who are resistant. Ecological factors involve physical, chemical, and biological environmental factors as well as the movement of mosquitoes from one area to another. The role of genetic factors in the occurrence of resistance is that mosquitoes naturally have

resistant genes that can undergo genetic selection when in contact with insecticides so that genetic diversity is formed. These resistant mosquitoes when mating with susceptible mosquitoes can produce resistant offspring so that the population of vectors with resistant traits will be greater (Metcalf and Luckman, 1975; Fuadzy and Hendri, 2015).

Research on genetic crosses between temephos-resistant strains and susceptible strains shows the inheritance of temephos resistance in *Aedes aegypti* mosquitoes occurs semi dominantly and as a monofactorial trait. Est-A₄ activity observed in polyacrylamide gel electrophoresis and measured by biochemical assays was higher in temephos-resistant strains, and lower in susceptible strains, and intermediates in crosses of the two strains. Retro crossing observations showed a lower effect on mortality with temephos as well as on Est-A₄ activity from resistant parent strains. These results suggest that esterase activity can also be inherited, as can resistance to temephos, as a semidominant character (Rodríguez et al., 2006). Other studies also showed that the inheritance of temephos resistance has a degree of dominance (D) value and the location of the F₁ line against the resistance line, this condition shows that the inheritance of temephos resistance in these species is monogenic and not completely dominant (Shetty, Sanil and Shetty, 2015).

In addition, there is a mechanism of metabolic resistance caused by esterase enzymes, quantitatively and qualitatively. Quantitatively, resistance occurs due to the production of too much esterase enzyme caused by concomitant changes in gene expression or co-amplification of esterase genes $\alpha 2$ (*est\alpha 2*) and $\beta 2$ (*est\beta 2*). While qualitatively resistance occurs due to an increase in the speed of enzyme activity in the hydrolysis of organophosphate insecticides. In addition to these two esterase genes, other genes that can cause resistance are monooxygenase and glutathione s-transferase. The presence of these genes causes the speed of detoxification of organophosphate insecticides to be faster because the more amplified genes cause the higher level of resistance. The higher level of resistance is due to insecticides being more reactive with esterase than their targets. If the concentration of esterase is comparable to that of an insecticide, it can inhibit the insecticide from the body's metabolic system and hydrolyze it into a nontoxic product. There are two mechanisms of enzyme changes that cause resistance, namely excessive production that increases insecticide metabolism and changes in the catalytic properties of enzymes to hypercatalytic (Lesmana et al., 2022).

An increase in the number of larvae in containers that have been intervened can also be caused by the possibility of inherited resistance in *Aedes* spp. mosquito eggs that hatch in those water containers. Basically, temephos can cause morphological abnormalities of *Aedes aegypti* eggs. This causes the egg to become flattened, the outer shell of the egg is more fragile so that the egg is easily cracked and even broken and cut (Yulidar, 2014). In a study in Cuba, it was reported that resistance to insecticides is related to genetic and bioecological factors. Based on biochemical tests, the study showed an increase in esterase which is closely related to temephos resistance (Yulidar, 2014). Resistant insects will create resistant hereditary so that the proportion of resistant vectors will increase in the population (Ikawati, Sunaryo and Widiastuti, 2015).

The vulnerability status of vectors can vary depending on the diversity of risk factors that cause resistance. The existence of this diversity causes differences in the development of resistance in each individual even though they come from the same species. Genetic diversity and environmental adaptability affect the tolerance level of each individual mosquito (Fuadzy and Hendri, 2015). Temephos has been used in Indonesia since 1976, and by 1980 had been used en masse as a Dengue control program (Istiana, Heriyani and Isnaini, 2012) The use of insecticides in a long period of time and high frequency can cause resistance (Lesmana et al., 2022). In a study conducted in Peru, there were differences in the level of resistance to temephos even after the use of larvicide was stopped. Rotational use of new larvicides with different ways of working can lower the incidence of larvicide resistance (Palomino et al., 2022).

4.2. Effect of Temephos on *Aedes* spp. larvae

There was an increase in the number of larvae-free houses in Bengkulu City. In the larvae survey before temephos intervention, 450 houses were found to be larvae-free, while after temephos intervention 556 houses were found to be larvae-free out of a total of 670 houses inspected. This study shows that the use of temephos as a non-systemic organophosphorus insecticide has an influence on the presence of *Aedes* spp. larvae in Bengkulu City. Temephos works by inhibiting the performance of the enzyme acetylcholinesterase AChE. Acetylcholinesterase is involved in the irreversible hydrolysis of the neurotransmitter acetylcholine which is a neurotransmitter involved in many cholinergic pathways in the body, which are the central nervous system and some aspects of the peripheral nervous system such as the somatic nervous system that innervates skeletal muscles as well as the sympathetic and parasympathetic autonomic nervous systems. Inhibition of AChE causes acetylcholine accumulation in synapses and neuromuscular links in cholinergic pathways resulting in hyperstimulation, restlessness, convulsions, muscle paralysis, tremors and death due to respiratory failure in larvae (Gan et al., 2021).

These mosquitoes generally breed in water reservoirs that do not touch the ground, such as water drums, bathtubs, flower vases, jars, used items that can hold rainwater in both urban and sub-urban areas (Wahyuni, Makomulamin and Sari, 2021). Adult mosquitoes will look for watery places to lay their eggs. In the research of Dom et al. (2016) showed that the most efficient mosquito breeding sites are shelters made from ceramics, glass cups and plastic bags filled with water (Dom et al., 2016).

The larvae of *Aedes* spp. could move agile and active in water containers. Larvae obtain food at the bottom of water containers, so they are called bottom feeders or basic food eaters. Therefore, the habit of the larvae becomes one of the supporting factors of the entry of temephos into the body of the larvae (Khaer, Kasim and Budirman, 2021). The penetration of temephos into the body of the larva takes place in a fast time and 99% of temephos is absorbed into the body of the larva within 24 hours after treatment (Rodríguez et al., 2002). Upon contact with temephos, the larvae will die and the residual effects of temephos will work to prevent the development of mosquitoes on the water container for three months (Kurniawan, Nurjana and Srikandi, 2019).

According to WHO (2009) there are two formulations that comply with specifications for quality control and international trade, which are emulsion concentration (EC) and granule (GR) formulations (World Health Organization, 2009). Only granule formulations can be used as mosquito larvicide in water, especially drinking water, at a dose not exceeding 1 mg/l of active ingredient (World Health Organization, 2009) Temephos at a dose of 1% has been proven to be effective for 8 - 12 weeks in normal water use patterns (World Health Organization, 2011). Based on research by Rosmini et al. (2006) stated that in the Palu City area the use of temephos can increase LFR and reduce the risk of dengue fever (Rosmini et al., 2006). This larvicide can paralyze the respiratory muscles of mosquito larvae, thereby increasing the larvae-free rate and reducing the incidence of dengue fever (Sumantri, 2014). Therefore, controlling the *Aedes aegypti* has a very big role in preventing dengue fever (Sukohar, 2014; Triana, Umniyati and Mulyaningsih, 2019; Triana et al., 2023)

5. Conclusion

In this study, from 67 urban-villages, in 44 (65,67%) urban-villages was found an increase of LFR after temephos intervention. The average LFR before temephos intervention was 67.61%, while after temephos intervention increased to 82.98%. In the test using Paired Sample T-Test, it was found that there was a significant difference between LFR of *Aedes* spp. before and after temephos intervention in Bengkulu City. Temephos intervention is effective to increase LFR of *Aedes* spp. in Bengkulu City. This study can still be developed to focused on testing the resistance of temephos. Although the overall use of temephos provides

a significant difference to LFR, considering that there are several urban villages that are at risk of resistance, it is important to conduct resistance test against temephos and monitoring other types of larvicide in Bengkulu City.

Rotation of the use of new larvicides with different modes of action can reduce the incidence of larvicidal resistance. The local governments need to conduct resistance tests on each larvicide that will be used for vector control. If the use of one larvicide decreases susceptibility and the possibility of resistance, then larvicide rotation is needed, namely using another type of larvicide to prevent resistance and increase LFR in an area.

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