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First report of the effect of lambda-cyhalothrin on the activity of acetylcholinesterase, glutathione S-transferase, and cytochrome C-oxidase enzymes in larvae of *Culex quinquefasciatus* (Diptera: Culicidae) in East Jakarta

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Abstract Bancroftian filariasis is a vector-borne disease transmitted by the *Culex quinquefasciatus*. Heavy and long-term use of insecticides causes the development of insecticide resistance in *Cx. quinquefasciatus*. However, there has been no research on resistance mechanisms through detoxifying enzyme activity in *Cx. quinquefasciatus* mosquitoes exposed to lambda-cyhalothrin in Jakarta. This study aimed to determine the larvicidal activity of lambda-cyhalothrin against *Cx. quinquefasciatus* larvae and the larvicidal mechanism through detoxifying enzyme activity. Bioassay tests were performed by exposing *Cx. quinquefasciatus* larvae to five concentrations of lambda-cyhalothrin (0.002; 0.015; 0.05; 0.2; 0.7 ppm). The mortality rate was measured after 24 h of exposure. The detoxifying enzyme activity, including acetylcholinesterase (AChE), glutathione s-transferase (GST), and cytochrome c-oxidase (COX), was analyzed using the biochemical method. Lambda-cyhalothrin (0.7 ppm) showed 100% mortality of *Cx. quinquefasciatus* larvae. LC_{50} dan LC_{90} values were 0.054 and 0.148 ppm, respectively. Lambda-cyhalothrin non-significantly increased AChE activity ($P > 0.05$), significantly increased GST activity ($P < 0.05$), and non-significantly decreased COX activity ($P > 0.05$). Lambda-cyhalothrin is still effective in killing *Cx. quinquefasciatus* larvae by influencing detoxification enzymes.

Keywords: *Culex quinquefasciatus*, detoxification enzyme, lambda-cyhalothrin

1. Introduction

Culex quinquefasciatus is known as the southern house mosquito and is one of the mosquitoes that are often found in various tropical and subtropical countries, including Indonesia (Negi and Verma 2018). *Cx. quinquefasciatus* serves as the main vector of St. Louis Encephalitis virus, West Nile virus, Western Equine Encephalitis virus, and bancroftian filariasis (Negi and Verma 2018; Lopes et al 2019). Approximately 13 billion people in the world in more than 83 countries are at risk of contracting filariasis or elephantiasis and 60% of cases are in Southeast Asia (Cogan 2021). In 2019, there were 10,758 cases of filariasis in Indonesia spread across 34 provinces (Hardhana et al 2019).

There are four classes of synthetic insecticides that are often used to control vector-borne disease (VBD) transmission, including pyrethroids (deltamethrin, lambda-cyhalothrin, and permethrin), organochlorines (DDT), carbamates (propoxur), and organophosphates (temephos and malathion). Control measures to avoid the spread and transmission of VBD are one of the most important steps in a VBD control and management program. These insecticides are widely used to control mosquito vectors, including *Cx. quinquefasciatus*. However, the use of insecticides that are not controlled and not managed properly causes the development of mosquito populations that are resistant to these insecticides. This can affect the efficacy of the insecticides used (Rai et al 2019). Gray et al (2018) proved that excessive use of household insecticides contributed to the occurrence of *Aedes aegypti* resistance to pyrethroids. In the northern part of West Bengal, India, population resistance of *Cx. quinquefasciatus* against synthetic pyrethroid insecticides has been reported (Rai et al 2019). Based on the literature analysis,

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until now, in Jakarta there have been no reports on the status of lambda-cyhalothrin resistance in *Cx. quinquefasciatus* mosquito.

Type II pyrethroids such as λ-cyhalothrin have an additional cyano group on the benzylic carbon atom which makes type II pyrethroids more potent (Ramchandra et al 2019). The main target of pyrethroids is the voltage-gated sodium channel (VGSC) in the nerve membrane (Lopes et al 2019). Pyrethroids modify the characteristics of VGSCs by slowing their closure. This causes a continuous influx of sodium ions (Na+) (referred to the sodium "tail current"), there by lowering the action potential threshold (Ramchandra et al 2019). The interaction results in the repeated firing of nerve impulses causing the insect to experience involuntary muscle spasms, fatigue, paralysis, and death. This phenomenon is known as the "knockdown effect" (Lopes et al 2019).

Target site insensitivity is one of the well-understood mechanisms of resistance (Rai et al 2019). However, another mechanism is related to *Cx. quinquefasciatus* resistance against lambda-cyhalothrin has not been widely studied (Zhang et al 2021). The mechanism of resistance can be evaluated not only based on DNA mutations to insecticides, but can also be based on the activity of detoxifying enzymes, such as acetylcholinesterase (AChE), glutathione s-transferase (GST), and cytochrome c-oxidase (COX) (Muthusamy and Shivakumar 2015; Brogdon 2014).

Previous research in India reported *Cx. quinquefasciatus* has been resistant to lambda-cyhalothrin with the mechanism of increasing glutathione reductase and esterase (Muthusamy and Shivakumar 2015). However, there has been no research on resistance mechanisms through detoxifying enzyme activity in *Cx. quinquefasciatus* mosquitoes exposed to lambda-cyhalothrin in Jakarta. This study aimed to determine the larvicidal activity of lambda-cyhalothrin against *Cx. quinquefasciatus* larvae in Jakarta and the larvicidal mechanism through detoxification enzyme activity such as AChE, GST, and COX activity.

2. Material and Methods

2.1. Ethical approval

The study was approved by the Ethics Committee from the Faculty of Medicine, the University of Indonesia (KET-048/UN2.F1. D1.2/PDP.01/Riset-2/2021).

2.2. Insecticides

This study used lambda-cyhalothrin purchased from a chemical store in Jakarta. There were five concentrations of lambda-cyhalothrin, namely 0.002, 0.015, 0.05, 0.2, and 0.7 ppm.

2.3. Cx. quinquefasciatus larvae collection sites

Cx. quinquefasciatus larvae were collected from East Jakarta, namely Kampung Gedong Subdistrict (Figure 1). Using a dipper, the larvae were collected from polluted stagnant water bodies, sewers, or drains surrounding houses, i.e., their natural habitat. They were washed with tap water in 2000 mL plastic containers before being placed in 1000 mL plastic containers containing tap water. All of the specimens were subsequently identified at the Entomology Laboratory of the Department of Parasitology, University of Indonesia. Only third and fourth in stars larvae were used in the larval bioassays.

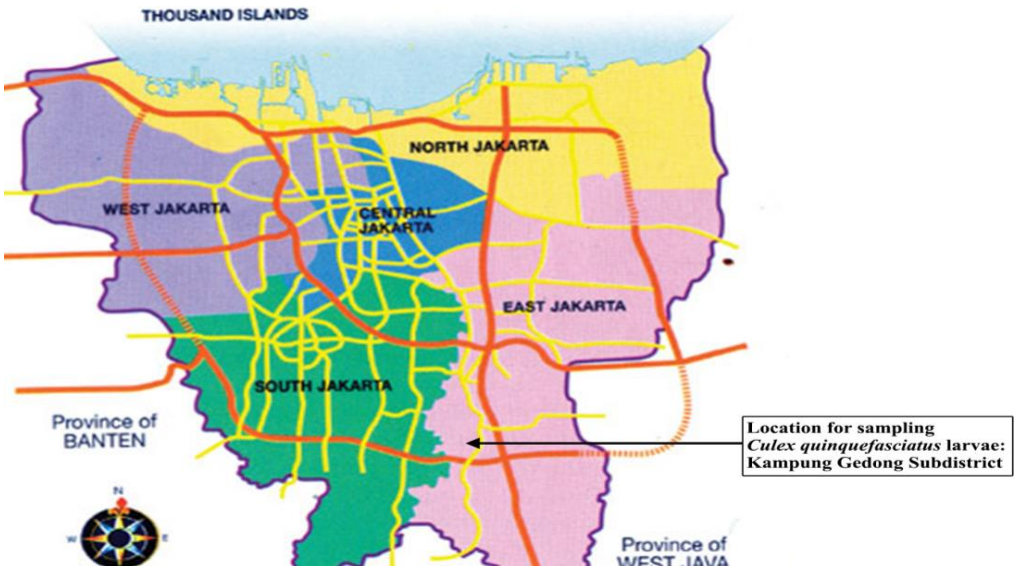

Figure 1 Map of location research.
Source: latitudes.nu

2.4. Larval susceptibility tests

Cx. quinquefasciatus larvae bioassay was carried out by the WHO protocol (WHO 2005; Dua et al 2013). In the control groups, a total of 25 healthy *Cx. quinquefasciatus* larvae were exposed only to tap water in a 200 mL plastic cup; five replicates were conducted using a total of 125 larvae. In the experimental treatment groups, 25 *Cx. quinquefasciatus* larvae per 200 mL plastic cup were exposed to five different concentrations of lambda-cyhalothrin, namely 0.002, 0.015, 0.05, 0.2, and 0.7 ppm. (a total of 625 larvae). After 24 h number of dead and live larvae was recorded.

2.5. Detoxifying enzyme activity

In this study, the number of larvae for examination of detoxification enzyme activity was 50 *Cx. quinquefasciatus* larvae from the control, temephos, malathion, cypermethrin, and deltamethrin groups respectively. So, the total larvae used was 250 larvae. *Cx. quinquefasciatus* larvae were used for the enzyme activity examination. The same samples were used to examine AChE, GST, and COX activity.

2.5.1. AChE activity

The AChE activity was assayed as previously described (Brogdon, 2014; Subahar *et al*, 2021). Dead larvae collected from the bioassays after the 24 h exposure to tested insecticides were homogenized in 1.0mL 0.25M KPO_4 (pH7.2). At room temperature, 100µL aliquots of the test sample homogenates were loaded into triplicate ELISA microplate wells. Similarly, 100 µL of the control (healthy *Cx. quinquefasciatus* larvae) solutions were added to triplicate microplate wells. Acetylcholine iodide (ATCh) and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were then added to every well (100 µL of each per well) and the absorbance at 414 nm was immediately read with an ELISA reader (T_0) and again after 10 minutes (T_{10}). The unit of AChE activity is absorbance per minute or Abs/min. Elisa reader made in Finland, Thermo Fisher, Scientific^{TM}, Cat number 51119000.

2.5.2. GST activity

GST activity was assayed as previously described (Brogdon 2014; Subahar et al 2021). The present study, the dead *Cx. quinquefasciatus* larvae collected from the bioassay after 24h exposure to tested insecticides were homogenized in 1.0 mL 0.25M KPO_4 (pH 7.2). Triplicate 100 µL aliquots of each homogenate were loaded into ELISA microplate wells at room temperature; similar wells were prepared using 100 µL of the control group (healthy *Cx. quinquefasciatus* larvae). Aliquots (100 µL) of reduced glutathione solution (Sigma G4251) and 1-chloro-2,4'-dinitrobenzene (cDNB) were added and the plates were read immediately at 340 nm (T_0) with an ELISA reader and again at 5 min (T_5). The unit of GST activity is absorbance per minute or Abs/min.

2.5.3. Cytochrome and COX activity

Cytochrome and COX activity were assayed as previously described (Brogdon 2014; Subahar et al 2021). The dead *Cx. quinquefasciatus* larvae were collected from the bioassays after 24 h exposure to tested insecticides. The dead *Cx. quinquefasciatus* larvae were homogenized with 1000 µL 0.25 M KPO_4 (pH 7.2). The following positive controls were also prepared: (i) 1:55 (22 µL stock, 1.2 mL KPO_4 buffer) and (ii) 1:110 (11 µL cytochrome stock, 1.2 mL KPO_4 buffer). Triplicate aliquots (100 µL) of the test sample homogenates were added to ELISA microplate wells, and 100µL KPO4 was added to the negative and positive control wells. The cytochrome-C positive control (cytochrome-c bovine heart) was added (100 µL), followed by a 200 µL TMBZ solution. One drop of 3% hydrogen peroxide (H_2O_2) was added to each well and incubated for 5 min. The plates were immediately read (T_0) at 620 nm with an ELISA reader. The unit of COX activity is absorbance per minute or Abs/min.

2.6. Data analysis

In this study, the data were analyzed using SPSS software (ver. 26). The lethal concentrations of 50% (LC_{50}) and 90% (LC_{90}) of lambda-cyhalothrin were calculated by probit analysis (Gomez and Anacta 2020). To find out whether there was a difference in the mortality rate of *Cx. quinquefasciatus* larvae between lambda-cyhalothrin concentrations, the one-factor ANOVA test was used if the data were normally distributed (Wang et al 2017). If the data is not normally distributed then the Kruskal-Wallis test (non-parametric statistics) is used (Guo et al 2013).

3. Results

3.1. Mortality percentage of Cx. quinquefasciatus larvae

The number of *Cx. quinquefasciatus* larvae successfully carried out the bioassay test on as many as 650 larvae consisting of 25 for the control group and 625 for the treatment group. The results of the bioassay test for 24 h are summarized in Table

1. In the control group, the mortality rate was 0% (all larvae survived). The lowest mortality of 28.8% was found at a concentration of 0.002 ppm and the highest of 100% was found at a concentration of 0.7 ppm.

The normality test using the Shapiro-Wilk test obtained data on the mortality rate of *Cx. quinquefasciatus* larvae had an abnormal distribution ($P < 0.05$ at 0.015 and 0.2 ppm lambda-cyhalothrin concentrations). The data was then transformed, but the results of the data distribution were still not normal, so it was continued with the Kruskal-Wallis non-parametric test. The results of the Kruskal-Wallis test showed that there was a significant difference in the mortality rate of *Cx. quinquefasciatus* larvae (bound variable) between groups of lambda-cyhalothrin concentration (independent variable). The post-hoc Tukey test showed that the mortality rate between the concentration groups was significantly different except at concentrations of 0.002 with 0.015 ppm (Z = -1.277, $P = 0.202$ ($P > 0.05$)) and 0.2 with 0.7 ppm (Z = -1.941, $P = 0.136$ ($P > 0.05$)).

Table 1 Mortality percentage of *Cx. quinquefasciatus* after expose to lambda-cyhalothrin for 24 h.

Treatment	N	Dead larvae		Median	Minimum	Maximum	*P*-value
		N	%				
Control	25	0	0,00%	-	-	-	-
lambda-cyhalothrin (ppm)							
0,002	125	36	28,8	7	3	10	0.000
0,015	125	45	36	8	8	11	
0,05	125	77	61,6	15	13	18	
0,2	125	121	96,8	25	25	25	
0,7	125	125	100	25	25	25	

P-value was calculated from Kruskal-Wallis Test.

LC_{50} and LC_{90} values of lambda-cyhalothrin on *Cx. quinquefasciatus* larvae are presented in Table 2. LC_{50} and LC_{90} values of lambda-cyhalothrinwere were 0.054 ppm (95% CI 0.038 – 0.068) and 0.148 ppm (95% CI 0.117 – 0.208) respectively. The results of the Probit analysis showed data on the mortality rate of *Cx. quinquefasciatus* larvae were in accordance with the Probit model used (*P*-value of Pearson Goodness-of-Fit Test > 0.05) and there was a significant difference in the mortality rate of *Cx. quinquefasciatus* larvae between groups of lambda-cyhalothrin concentrations (*P*-value of regression <0.05).

Table 2 Lethal concentration (LC) of lambda-cyhalothrin.

Insecticide	LC_{50} (ppm)	95%Cl	LC_{90} (ppm)	95%Cl	SE	Z	*P*-value (Regression)	*P*-value (PGFT)
Lambda-cyhalothrin	0,054	0,038-0,068	0,148	0,117-0,208	0,461	6,315	0,000	0,791

LC: Lethal concentration; CI: Confidence interval; SE: Standard error; PGFT: Pearson Goodness-of-Fit Test

3.2. Detoxification enzyme activity on Cx. quinquefasciatus larvae

The number of larvae used for the control group was 25 larvae and the larvae were divided into three Eppendorf tubes (n1 = 10, n2 = 10, n3 = 5). For the treatment group, 404 larvae were used and the larvae were divided into 5 Eppendorf tubes according to their respective concentrations: concentration of 0.002 ppm (n = 36), 0.015 ppm (n = 45), 0.05 ppm (n = 77), 0.2 (n = 121), and 0.7 ppm (n = 125).

The control group showed an increase in enzyme activity of AChE (0.0636±0.0012 Abs/min), GST (0.054±0.0003 Abs/min), and COX (0.007±0.0002 Abs/min). The lambda-cyhalothrin group with a concentration of <0.7 ppm (0.002, 0.015, 0.05, and 0.2 ppm) showed an increase in the activity of the AChE enzyme (0.0033±0.0025 Abs/min), GST (0.0051±0.0010 Abs/min), and COX (0.0005±0.0004 Abs/min). While in the lambda-cyhalothrin group at a concentration of 0.7 ppm, it showed an increase in the activity of AChE enzymes (0.0005±0.0001 Abs/min) and GST (0.0024±0.0003 Abs/min), but there was a decrease COX enzyme activity (-0.0038±0.0002 Abs/min), is shown in Figure 2.

The results of the Shapiro-Wilk normality test found that AChE and GST enzyme activity data were normally distributed ($P > 0.05$). In contrast to the COX enzyme data, which showed an abnormal distribution of data ($P < 0.05$). The results of the paired T test on the lambda-cyhalothrin treatment (all concentrations) showed an insignificant increase in AChE enzyme activity (0 min and 10 min) ($P = 0.070$, $P > 0.05$), while the increase in GST enzyme activity (0 min and 5 min) is significant ($P = 0.002$, P

<0.05). Nonparametric Wilcoxon Signed-Rank Test for COX enzyme activity showed that COX enzyme activity (0 min and 5 min) was not significant ($P = 0.713$, $P > 0.05$). Overall (all concentrations), lambda-cyhalothrin exposure in *Cx. quinquefasciatus* larvae resulted in a non-significant increase in the activity of the AChE enzyme, a significant increase in the GST enzyme, and a non-significant decrease in the COX enzyme.

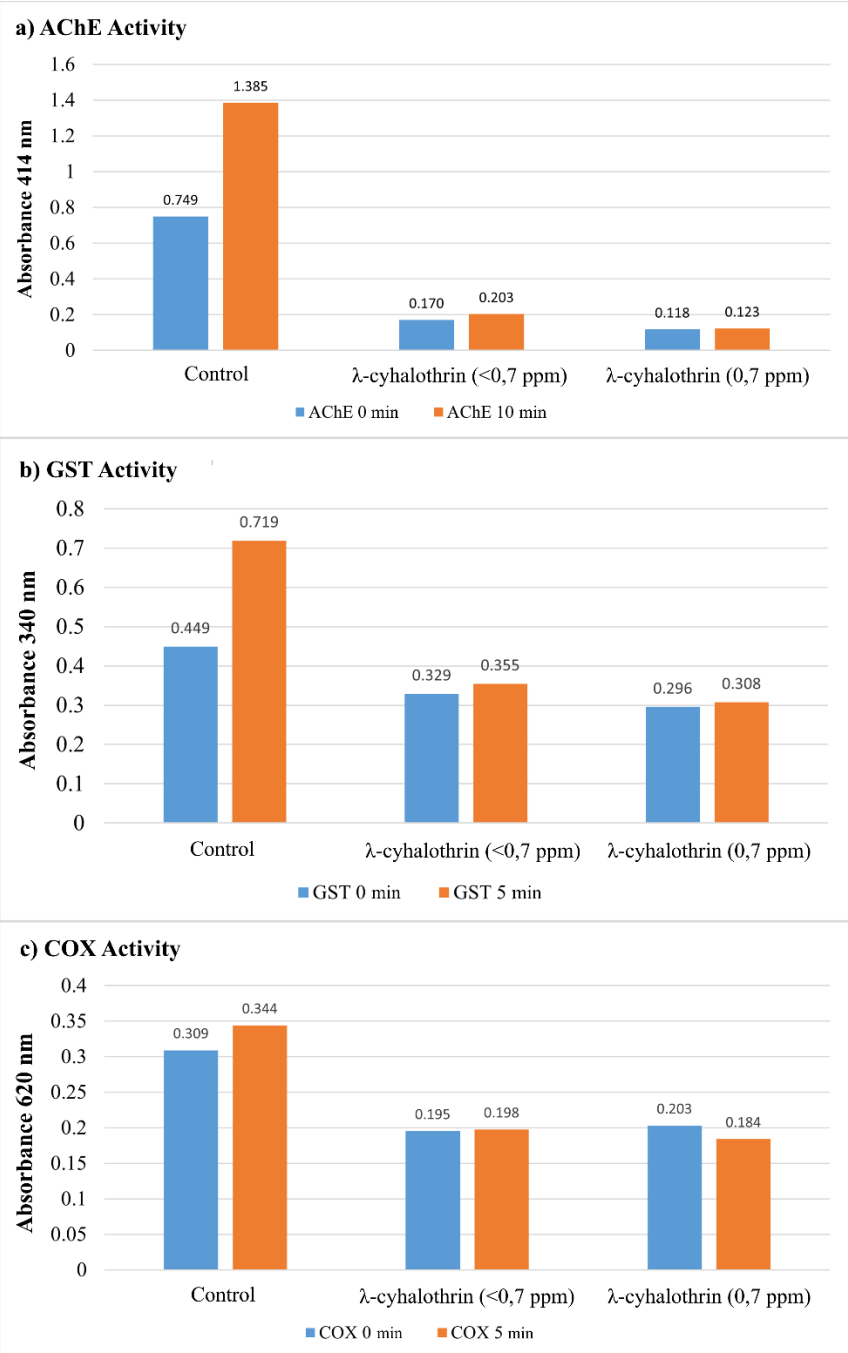

Figure 2 Activity (average absorbance) of AChE, GST, and COX enzymes on *Cx. quinquefasciatus* larvae (the control group, lambda-cyhalothrin concentration <0.7 ppm, and concentration 0.7 ppm). AChE enzyme activity was not significant ($P > 0.05$), GST was significant ($P < 0.05$), and COX was not significant ($P > 0.05$).

4. Discussion

Bancroftian filariasis is still a major health problem in the world and in Indonesia (Lee and Ryu 2019). One way to eradicate filariasis is by controlling *Cx. quinquefasciatus* mosquito using insecticides. Pyrethroid insecticides are commonly used in the community, including lambda-cyhalothrin. However, uncontrolled use of insecticides can lead to resistance in the mosquito population (Rai et al 2019). For example, Zanzibar has proven resistant to *Cx. quinquefasciatus* from Wete, Pemba Island against all classes of insecticides, including lambda-cyhalothrin (Jones et al 2012).

In this study, a 24 h bioassay test on lambda-cyhalothrin treatment found mortality of *Cx. quinquefasciatus* larvae increased with increasing concentration. The control group showed a mortality rate of 0% (0/25) indicating that there were no dead larvae of *Cx. quinquefasciatus*. This was because the control group was given tap water that did not have an active substance to kill the larvae.

The treatment of lambda-cyhalothrin at concentrations of 0.002, 0.015, 0.05, 0.2, and 0.7 ppm resulted in mortality of 28.8% (36/125), 36% (45/125), 61.6% (77 /125), 96.8% (121/125), and 100% (125/125). The results of the Kruskal-Wallis nonparametric test showed that there was a significant difference in the mortality rate of *Cx. quinquefasciatus* between groups of lambda-cyhalothrin concentrations ($p < 0.05$). The post-hoc Tukey test showed that there was a significant difference in the mortality rate of *Cx. quinquefasciatus* larvae between groups of lambda-cyhalothrin concentrations except at concentrations of 0.002 and 0.015 ppm and concentrations of 0.2 and 0.7 ppm. This phenomenon was caused by the higher concentration of lambda-cyhalothrin containing more active substances so that the ability of *Cx. quinquefasciatus* larvae to eliminate lambda-cyhalothrin decreases with increasing concentration and causes an increase in mortality (Lushchak et al 2018).

In contrast to the results of the study of Tmimi et al (2018) in Morocco who reported mortality of *Cx. pipiens* (Fam: Culicidae) adults by 49% after exposure to lambda-cyhalothrin at a concentration of 0.05% for 24 h. Rahimi *et al* (2019) in Iran also demonstrated mortality of *Cx. pipiens* adults by 40% after exposure to lambda-cyhalothrin with the same concentration and duration. The results of this study show that in other countries (Morocco and Iran), *Cx. pipiens* or *Cx. quinquefasciatus* has developed resistance to lambda-cyhalothrin with a concentration of 0.05% (WHO standard) (Tmimi et al 2018; Rahimi et al 2019). Meanwhile, in Jakarta using lambda-cyhalothrin with a concentration of 0.7 ppm was proven to be still effective in killing *Cx. quinquefasciatus* larvae and has a 100% mortality rate. This could be due to lambda-cyhalothrin which is still rarely used in Jakarta and the difference in a geographical area. The location of Jakarta which is far from rural areas and rice fields also causes the mosquito population to be less frequently exposed to insecticides including lambda-cyhalothrin, while in Iran (a city south of Tehran) and Morocco (outside urban areas) the use of lambda-cyhalothrin insecticides is high (Tmimi et al 2018; Rahimi et al 2019).

The effectiveness of lambda-cyhalothrin can also be assessed through the LC_{50} and LC_{90} values obtained from the Probit regression test. In this study, found LC_{50} of 0.054 ppm (95% CI 0.038 – 0.068) and LC_{90} of 0.148 ppm (95% CI 0.117 – 0.208). The study conducted by Emtithal et al (2012) reported that the larval population of *Cx. pipiens* from Sharkia Province, Egypt had LC_{50} and LC_{90} due to lambda-cyhalothrin of 0.072 ppm (95% CI 0.06 – 0.076) and 14,527 ppm (95% CI 12.04 – 17.01). Zhao et al (2014) reported that the larval strain of *Cx. quinquefasciatus* from seven different populations in China had LC_{50} due to lambda-cyhalothrin of 0.014 – 1.291 ppm. Differences in results with previous studies can be caused by geographical differences as environmental factors (susceptibility status) and the concentration range of insecticides used (Emtithal et al 2012; Zhao et al 2014).

This study also shows one of the susceptibility mechanisms, namely through the activity of detoxifying enzymes (AChE, GST, and COX). Lambda-cyhalothrin <0.7 ppm (0.002, 0.015, 0.05, and 0.2 ppm) and 0.7 ppm. This division is intended to make the analysis of enzyme activity more specific. In this study, the control group had higher AChE, GST, and COX enzyme activities than the treatment group. This indicates that there are many insecticide molecules that enter the larvae of the treatment group that must be detoxified by the larvae (Ahmad et al 2007). This study showed an increase in AChE activity, both in the control group (0.0636±0.0012 Abs/min), lambda-cyhalothrin concentration <0.7 ppm (0.0033±0.0025 Abs/min), and 0.7 ppm concentration (0.0005±0.0001 Abs/min). A study conducted by Low et al (2013) in Malaysia showed an increase in AChE enzyme activity in a population of *Cx. quinquefasciatus* mosquito.

5. Conclusions

This study showed the mortality rate of *Cx. quinquefasciatus* larvae after exposure to lambda-cyhalothrin for 24 h ranged from 28.8% to 100%. LC_{50} and LC_{90}values of lambda-cyhalothrin were 0.054 ppm (95% CI 0.038–0.068) and 0.148 ppm (95% CI 0.117–0.208). Lambda-cyhalothrin caused an insignificant increase in AChE enzyme activity ($P > 0.05$), a significant increase in GST enzyme activity ($P < 0.05$), and an insignificant decrease in COX enzyme activity ($P > 0.05$). Therefore, lambda-cyhalothrin (0.7 ppm) was still effective to kill *Cx. quinquefasciatus* larvae with the mechanism of influencing detoxification enzymes.

Conflict of Interest

The authors declared that there is no conflict of interest.

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References

Ahmad I, Astari S, Tan M (2007) Resistance of *Aedes aegypti* (Diptera: Culicidae) in 2006 to pyrethroid insecticides in Indonesia and its association with oxidase and esterase levels. Pakistan Journal of Biological Science 10:3688-3692.

Brogdon WG (2014) Microplate Enzyme Activity Assays. In: Benedict MQ (ed) Methods in Anopheles Research, 4th eds. Communicable Disease Control & Prevention, Atlanta, pp 236-247.

Cogan JE (2021) Lymphatic filariasis.World Health Organization. Accessed on: July 30, 2021. https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis

Dua VK, Kumar A, Pandey AC, Kumar S (2013) Insecticidal and genotoxic activity of *Psoralea corylifolia* Linn. (Fabaceae) against *Culex quinquefasciatus*Say, 1823. Parasites & Vectors 6:30-38.

Emtithal AES, Thanaa AEB (2012) Efficacy of some insecticides on field populations of *Culex pipiens* (Linnaeus) from Egypt. The Journal of Basic and Applied Zoology 65: 62-73.

Gray L, Florez SD, Barreiro AM, Vadillo-Sánchez J, González-Olvera G, Lenhart A, Manrique-Saide P, Vazquez-Prokopec GM (2018) Experimental evaluation of the impact of household aerosolized insecticides on pyrethroid resistant *Aedes aegypti*. Scientific Report 8:1-11.

Gomez DC, Anacta N (2020) A New Method to Test Molluscicides against the Philippine Schistosomiasis Snail Vectors. Journal of Parasitology Research 2020:3827125.

Guo S, Zhong S, Zhang A (2013) Privacy-preserving Kruskal-Wallis test. Computer Methods and Programs in Biomedicine 112:135-45.

Hardhana B, Sibuea F, Widiantini W (2020) Indonesia's 2019 health profile. Ministry of Healthof The Republic of Indonesia Jakarta (Indonesian language).https://pusdatin.kemkes.go.id/resources/download/pusdatin/profil-kesehatan-indonesia/Profil-Kesehatan-indonesia-2019.pdf

Jones CM, Machin C, Mohammed K, Majambere S, Ali AS, Khatib BO, McHa J, Ranson H, Kelly-Hope LA (2012) Insecticide resistance in *Culex quinquefasciatus* from Zanzibar: implications for vector control programmes. Parasites & Vectors 5:78-87.

Lee J, Ryu JS (2019) Current Status of Parasite Infections in Indonesia: A Literature Review. Korean Journal of Parasitology 57:329-339.

Lopes RP, Lima JBP, Martins AJ (2019) Insecticide resistance in *Culex quinquefasciatus* Say, 1823 in Brazil: a review. Parasites & Vectors 12:591-606.

Low VL, Chen CD, Lee HL, Tan TK, Chen CF, Leong CS, Lim YA, Lim PE, Norma-Rashid Y, Sofian-Azirun M (2013) Enzymatic characterization of insecticide resistance mechanisms in field populations of Malaysian *Culex quinquefasciatus* Say (Diptera: Culicidae). PLoS One. 8:e79928-e79936.

Lushchak VI, Matviishyn TM, Husak VV, Storey JM, Storey KB (2018) Pesticide toxicity: a mechanistic approach. EXCLI J17:1101-36.

Muthusamy R, Shivakumar MS (2015) Effect of lambda cyhalothrin and temephos on detoxification enzyme systems in *Culex quinquefasciatus* (Diptera: Culicidae). Journal of Environmental Biology 36:235-9.

Negi C, Verma P (2018) Review on *Culex quinquefasciatus*: southern house mosquito. International Journal of Life Science Scientific Research 4:1563-66.

Rahimi S, Vatandoost H, Abai MR, Raeisi A, Hanafi-Bojd AA (2019) Status of resistant and knockdown of West Nile vector*, Culex pipiens* complex to different pesticides in Iran. Journal of Arthropod Borne Disese13:284-96.

Rai P, Bharati M, Subba A, Saha D (2019) Insecticide resistance mapping in the vector of lymphatic filariasis, *Culex quinquefasciatus* Say from northern region of West Bengal, India. PLoS One 14:e0217706-e0217723.

Ramchandra AM, Chacko B, Victor PJ (2019) Pyrethroid poisoning. Indian Journal of Critical Care Medicine 23(Suppl 4): S267-271.

Salim-Abadi Y, Asadpour M, Sharifi I, Sanei-Dehkordi A, Gorouhi MA, Paksa A, Tayyebi Z, Aghaei-Afshar A (2017) Baseline susceptibility of filarial vector *Culex quinquefasciatus* (Diptera: Culicidae) to five insecticides with different modes of action in southeast of Iran. Journal of Arthropod Borne Disease 11:453-62.

Subahar R, Susanto L, Aidilla R, Aulia AP, Yulhasri Y, Winita R, Lubis NS, Sari IP (2021) *In vitro* experiments of *Pediculus humanus capitis* (*Phthiraptera*: *Pediculidae*) resistance to permethrin and 6-paradol in East Jakarta: Detoxification enzyme activity and electron microscopic changes in lice. Veterinary World14:3065-3075.

Tmimi FZ, Faraj C, Bkhache M, Mounaji K, Failloux AB, Sarih M (2018) Insecticide resistance and target site mutations (G119S ace-1 and L1014F kdr) of *Culex pipiens* in Morocco. Parasites & Vectors11:51-60.

Wang Y, Rodríguez de Gil P, Chen YH, Kromrey JD, Kim ES, Pham T, Nguyen D, Romano JL (2017) Comparing the Performance of Approaches for Testing the Homogeneity of Variance Assumption in One-Factor ANOVA Models. Educational and Psychological Measurement. 77:305-329.

World Health Organization (2005) Guidelines for laboratory and field testing of mosquito larvicides. WHO, Geneva. https://apps.who.int/iris/bitstream/handle/10665/69101/WHO_CDS_WHOPES_GCDPP_2005.13.pdf?sequence=1&isAllowed=y

Zhang C, Shi Q, Li T, Cheng P, Guo X, Song X, Gong M (2021) Comparative proteomics reveals mechanisms that underlie insecticide resistance in *Culex pipienspallens*Coquillett. PLoS Neglected Tropical Diseases 15:e0009237-e0009260.

Zhao M, Dong Y, Ran X, Guo X, Xing D, Zhang Y, Yan T, Zhu X, Su J, Zhang H, Wang G, Hou W, Wu Z, Li C, Zhao T (2014) Sodium channel point mutations associated with pyrethroid resistance in Chinese strains of *Culex pipiensquinquefasciatus* (Diptera: Culicidae). Parasites & Vectors7:369-79.