# First report of Culex quinquefasciatus Say (Diptera: Culicidae) larvae susceptible to temephos, malathion, cypermethrin, and Deltamethrin in Jakarta, Indonesia: Detoxification enzyme activity and the midgut cell damage

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## Abstract

**Background**: Insecticide-resistant *Culex quinquefasciatus* mosquitoes have been reported worldwide but the susceptibility status of *Cx.quinquefasciatus* larvae to insecticides has not been determined in Jakarta, Indonesia. This study aimed to evaluate the susceptibility status of *Cx. quinquefasciatus* larvae to temephos, malathion, cypermethrin, and deltamethrin in Jakarta, Indonesia, through the mode of action detoxification enzyme activity and digestive system damage.

**Methods***: Cx. quinquefasciatus* larvae were collected from five sites in Jakarta. The larval susceptibility test to temephos used the WHO standard kits (1.25, 6.25, 31.25, and 156.25 ppm) and to malathion (0.5 ppm), cypermethrin (.25 ppm), and deltamethrin (0.35 ppm). The activity of acetylcholinesterase (AChE), glutathione S-transferase (GST), and oxidase were measured by the biochemical method. The larval midgut cell damage was examined using the routine histopathological method and transmission electron microscopy (TEM).

**Results:** At 24 h, temephos and deltamethrin caused 100% larval death of *Cx.quinquefasciatus* in five sites of Jakarta. Temephos and malathion significantly inhibited AChE activity, while cypermethrin and deltamethrin significantly inhibited oxidase activity. All tested insecticides significantly damaged the larval midgut. The TEM findings showed the damaged parts of the midgut epithelial cells included the cell membrane, nuclei, nucleoli, mitochondria, and other organelles cells.

**Conclusion:** This study is the first to report that *Cx.quinquefasciatus* larvae in Jakarta were completely susceptible to temephos and deltamethrin through metabolic enzymes and midgut cell damage. This study provides baseline data to improve mosquito control programs.


## Background

Vector-borne diseases (VBDs) are still a globally public health problem [1, 2]. VBDs are transmitted by mosquitoes. *Culex quinquefasciatus* Say (Diptera; Culicidae), known as southern house mosquito, is an important vector that transmits VBDs consisting of Zika, Japanese encephalitis, West Nile Fever, Rift Valley Fever, and lymphatic filariasis [3–5]. *Cx.quinquefasciatus* populations are abundant and widespread distributed in subtropical and tropical countries [6, 7]. Additionally, it is a potential bridge between sylvatic arbovirus from birds to man in urban areas [5].

Two synthetic insecticide groups are frequently used to control mosquitoes such as organophosphate and pyrethroid [8, 9]. Organophosphates (OP) are esters of phosphoric acid and the mechanism of action of OP is the irreversible inactivation of the acetylcholinesterase enzyme [10–12]. The OPs have the property of being rapidly degraded by hydrolysis on exposure to light, air, and soil [10]. This study used temephos and malathion that the OP group frequently used to control mosquitoes in the world [8, 10]. Temephos is an effective insecticide to kill mosquito larvae [13], while malathion kills mosquito adults effectively [10].

The pyrethroid (PYR) group, is α-cyano pyrethroid and neurotoxic for insects [14]. PYR contains natural pyrethrin extracted from pyrethrum flowers and their synthetic derivates [15, 16]. Pyrethrins are derived from keto alcoholic esters of chrysanthemum and pyrethroid acids [8]. PYRs affect the sodium channels and cause paralysis of the organism [12]. Previous studies reported that cypermethrin and deltamethrin are widely used worldwide due to their valuable insecticidal activity against pests and parasites [14, 15]. Deltamethrin causes varying degrees of toxicity due to oxidative stress [14, 17] and it has been reported to be cytotoxic in human lung cells [18]. Thus, this study used cypermethrin and deltamethrin to evaluate in vitro susceptible status of *Cx.quinquefasciatus* larvae.

Many studies showed that mosquitoes have been resistant to insecticides in the world such as insecticide-resistant *Cx.quiquefasciatus* larvae and female mosquitoes [12, 19]. Two resistance mechanisms in mosquitoes are increased metabolic detoxification of insecticides and decreased sensitivity of the target protein on which the insecticide acts, which is called target site insensitivity [12, 20]. First, metabolic resistance involved monooxygenase (cytochrome P450), glutathione S-transferase (GST), and esterase (EST) [12, 21]. Second, target site insensitivity involved altered acetylcholinesterase (AChE), and knockdown resistance (*kdr*) of voltage gate sodium channel (VGSC) gene [22]. The activity of detoxification enzymes such as AChE, GST, EST, and oxidase activity, to evaluate the mechanism of insecticide-resistant mosquitoes have been reported [23, 24]. Increased monooxygenase and EST are associated with mosquito resistance to PYR, DDT, carbamate, and OP [25, 26], while increased GST is associated with mosquito resistance to DDT only. Point mutations of *kdr* gene are associated with mosquito resistance to PYR and DDT and altered AChE is associated with mosquito resistance to carbamate and OP [12, 20]. Also, elevated oxidase levels are associated with resistance to various insecticides classes [23]. Moreover, Oliver and Brook [27] have been demonstrated that insecticide-resistant individuals show an increased capacity to cope with oxidative stress, mediated by increased glutathione peroxidase and catalase activity.

Oxidative stress plays an important role in various toxicities associated with synthetic insecticides including OP and PYR [17]. For example, the toxic activity of PYR on non-target organisms, animal and human, showed widely effects such as neurotoxicity, immunotoxicity, cardiotoxicity, hepatotoxicity, reproductive, genotoxic, hematotoxicity effects, digestive system toxicity, and cytotoxicity [17]. Şekeroğlu, et al [18] demonstrated both the mixture of deltamethrin and thiacloprid and their metabolites significantly reduced cell viability and induced cytotoxicity in human lung fibroblast cells.

In Indonesia, in 1993, both *Anopheles koliensis* and *Cx.quinquefasciatus* in Irian Jaya (Papua) have been reported resistant to DDT [28]. Afterward, insecticide-resistant *Cx.quinquefasciatus* did not be reported in other provinces in Indonesia. Up to date, in Jakarta, Indonesia, the susceptibility status of *Cx.quinuqefasciatus* larvae to temephos, malathion, cypermethrin, and deltamethrin has not been established. Thus, the present study evaluated in vitro susceptible status of *Cx.quinquefasciatus* larvae collected from the five fields in Jakarta. The larval susceptibility test of *Cx.quinquefasciatus* to temephos used the WHO standard kits (1.25, 6.25, 31.25, and 156.25 ppm [13]. In other insecticides, we used low concentrations of malathion (0.5 ppm), cypermethrin (0.25 ppm), and deltamethrin (0.35 ppm) by modifying the temephos concentrations of the WHO standard kits [13]. This study demonstrated that temephos, malathion, cypermethrin, and deltamethrin caused changes in the activity of AChE, GST, and oxidase in *Cx.quiqnuefasciatus* larvae. To prove that temephos,

malathion, cypermethrin, and deltamethrin showed toxicity in the digestive system [17], the midgut damage of *Cx.quinquefasciatus* larvae exposed to the tested insecticides was observed by the routine histopathological method [29] and transmission electron microscopy (TEM) [30].

This study chose Jakarta, the capital city of Indonesia, for the study site because VBD cases such as malaria, dengue hemorrhagic fever (DHF), and filariasis are still found in Jakarta. For example, In Jakarta in 2019, there were 52 suspected cases of malaria, 8716 cases of DHF with an incidence rate of 83.0% per 100.000 population, 22 cases of chronic filariasis, and 1 new case of filariasis was found in East Jakarta [31]. In Jakarta, there are many breeding places for *Cx.quinquefasciatus* larvae around people's homes, such as sewers with dirty and foul-smelling water. This study aimed to evaluate in vitro susceptibility status of *Cx.quinquefasciatus* larvae to temephos, malathion, cypermethrin, and deltamethrin in Jakarta, Indonesia, through the mode of action of detoxification enzyme activity and digestive system damage especially damage to the larval midgut cells.

## Methods

### *Ethical approval*

The study was approved by the Ethics Committee from the Faculty of Medicine, the University of Indonesia (No.KET-055/UN2.F1.D1.2/PDP/Riset-2/2019).

### *Insecticides*

The study used two insecticides from the organophosphate group, malathion ($C_{10}H_{19}O_6PS_2$) and temephos ($C_{16}H_{20}O_5P_2S_3$), and two from the pyrethroid group, cypermethrin ($C_{22}H_{19}Cl_2NO_3$) and deltamethrin ($C_{22}H_{19}Br_2NO_3$). All the insecticides were purchased from chemical shops in Jakarta. These insecticides are registered in Indonesia as follows: malathion: MEGATHION 1200 UL, PT Citra Sari Kimia, No. Reg. RI 0809120103771; deltamethrin: DELFOX 25 EC, PT Indo Pest Biochem, No. Reg. RI 06090120093424; cypermethrin: CYNOFF, PT Bina Guna Kmia, However, liquid temephos was purchased from the Directorate of Vector-Borne and Zoonotic Diseases, Ministry of Health Indonesia, Jakarta, Indonesia.

### *Cx. quinquefasciatus larvae collection sites*

*Cx. quinquefasciatus* larvae were collected from five fields of Jakarta; 1) Kampung Gedong (East Jakarta), 2) Johar Baru (Central Jakarta), 3) Marunda (North Jakarta), 4) Cengkareng (West Jakarta), and 5) Setu Babakan (South Jakarta) as seen in Fig 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 instars larvae were used in the larval bioassays.

In each study site, Research Team recorded the use of insecticides by the health center, natural breeding sites for *Cx. quinquefasciatus* mosquito, and household insecticides in supermarkets. The number of cases of malaria, DHF, and filariasis in Jakarta was obtained from the annual report (2019) of the Jakarta Health Office [31].

### *Larval Susceptibility tests*

In this study, the larval susceptibility test was divided into two parts. First, the larval susceptibility test to temephos used the WHO standard kits (1.25, 6.25, 31.2 In the second part, we used malathion (0.5 ppm), cypermethrin (0.25 ppm), and deltamethrin (0.35 ppm) concentrations by modifying the temephos concentration of the WHO standard kits [13,15].

*Cx.quinquefasciatus* larvae bioassay was carried out by the WHO protocol [14]. 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 each of the tested four insecticides (a total of 625 larvae). After 24 h of exposure in each experimental group, the number of dead and live larvae were recorded.

### *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 were 250 larvae. *Cx.quinquefasciatus* larvae were used for the enzyme activity examination. The same samples were used to examine AChE, GST, and oxidase activity.

### *AChE activity*

The AChE activity was assayed as previously described [23,24]. Dead larvae collected from the bioassays after the 24 h exposure to tested insecticides were homogenized in 1.0 mL 0.25 M $KPO_4$ (pH 7.2). At room temperature, 100 µL 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 (ACTH) 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™, Cat number 51119000.

## GST activity

GST activity was assayed as previously described [23.24]. The present study, the dead *Cx. quinquefasciatus* larvae collected from the bioassay after 24 h exposure to tested insecticides were homogenized in 1.0 mL 0.25 M $KPO_4$ (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.

## Oxidase activity

Oxidase was assayed as previously described [23,24]. 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, µL 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 100 µL 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 5min. The plates
were immediately read ($T_0$) at 620 nm with an ELISA reader. The unit of oxidase activity is absorbance per minute or Abs/min.

## Toxicity of OP and PYR

A routine histopathological technique was employed as previously described [29]. In total, 150 dead *Cx. quinquefasciatus* larvae were examined; these consisted of 25 larvae from the negative control group and 125 larvae from the treatment groups (25 larvae from
each insecticide treatment). All of these specimens were fixed with 10% formalin and then dehydrated using a series of increasing alcohol concentrations (70%, 80%, 90%, 95%, and 100%). Afterward, the specimens were embedded in xylene 1, xylene 2, and xylene 3 solutions and in paraffin blocks.
The blocks were cut to thicknesses of 5 µm using a manual microtome (Model 320, No. 17664,
USA) and feather microtome blades (Feather, S35, Japan). Finally, the sections were stained with hematoxylin and eosin and the stained specimens were obse
light microscope and imaged using a digital microscopic mounted camera (Zeiss Axiocam ERC 5s, Germany).

The present study used TEM to evaluate the damage of midgut cells including cell membrane, mitochondria, and others. The samples were processed according to Ma et al [30], with a slight modification of the fixation liquid. The whole bodies of the treated *Cx. quinquefasciatus* larvae were pre-fixed in 2.5% glutaraldehyde at 4°C for a minimum of 2 days and then washed three times with cacodylate buffer for 15 min each time. The samples were fixed in 2% osmium tetroxide and 2.5% $K_3Fe(CN)_6$ in the buffer for 2 h, and then rinsed in cacodylate buffer as described in the previous step. The samples were then dehydrated in an ethanol series in ascending order (30%, 50%, 70%, 80%, 90%, and 100%) for 15 min each. After dehydration, the samples were infiltrated using absolute ethanol and propylene oxide in specific ratios (2:1, 1:1, 1:2, v/v) for 30 min each. The samples were embedded in Spurr resin. The prepared samples were cut using an ultramicrotome (Leica UC6, Wetzlar, Germany) and observed using TEM (JEOL JEM 1010, Japan).

## Data analysis

Data were analyzed using Statistical Package for Social Science (SPPS) version 26.0 The susceptibility response of the *Cx.quinquefasciatus* larvae to the insecticides was determined based on criteria as previously described [32]. At 24 h, a 100% death rate indicated that *Cx.quinquefasciatus* larvae were completely susceptible to the insecticide, a 99-90 % death rate indicated moderate susceptibility, and a < 90% death rate indicated low susceptibility [32].

Data from AChE (0 min to 10 min), GST (0 min to 5 min), oxidase (0 min to 5 min) activities were not a normal distribution, so the Wilcoxon Signed Ranks Test (nonparametric) was used [33]. The purpose of the Wilcoxon tests was to determine whether there was a difference in the mean AChE activity from 0 min to 10 min, GST activity from 0 min to 10 min, and oxidase activity from 0 min to 5 min.

The digestive system cytotoxicity of tested insecticides was measured based on damage to the midgut of *Cx.quinquefasciatus* larvae including the breakdown of the epithelial cells, microvilli, peritrophic membranes, and food boluses. Because the midgut damage data were not normally distributed, the Kruskal-Wallis test was used [34]. The purpose of the Kruskal-Wallis test was to determine whether there was a significant difference in midgut damage due to exposure to the insecticide tested.

## Results

Table 1 shows the description of VBD cases, mosquito control, usage of insecticides, usage of insecticides, and natural breeding places in Jakarta. VBD cases consisted of malaria, DHF, and filariasis. DHF cases were found to be the most common compared to malaria and filariasis in all areas of Jakarta. Mosquito eradication activities are prioritized on eradicating *Aedes sp*, DHF vectors, while other mosquitoes are not carried out. The Jakarta Health Office actively uses

PYR (cypermethrin) to control DHF vectors. Furthermore, natural breeding places for *Anopheles sp* were only found in North Jakarta, but for *Aedes sp* and *Cx.quinquefasciatus* found in five study areas (Table 1). Fig 2 shows natural breeding places for *Cx.quinquefasciatus* larvae such as sewers in front of people's houses with no running water, dirty, lots of garbage, and a bad smell.

## *Mortality rate of Cx.quinquefasciatus*larvae

In this study as many as 4,375 *Cx.quinquefasciatus* larvae were successfully examined at five sampling sites. At each larval sampling site, as many as 875 larvae were examined. The larval mortality rates of each tested insecticide are summarized in Table 2. In the temephos group, all *Cx quinquefasciatus*larvae died after exposure to temephos from low to high concentrations in the five sampling areas. The same results were found in the deltamethrin group. Overall, the larval mortality rate for temephos and deltamethrin was 100% in all sampling sites.

In the malathion group, the larva mortality rate of 100% was found at the first sampling location, East Jakarta. The larval mortality rate ranged from 96.8% to 100% (Table 2). In the cypermethrin group, the mortality of *Cx quinquefasciatus*larvae ranged from 90.4% to 100%. A larval mortality rate of 100% was found at the third sampling location, North Jakarta. Overall, the results of this study found complete susceptibility of *Cx quinquefasciatus*larvaeto temephos and deltamethrin atfivesamplingsites(Table2).

## *Detoxification enzyme activity*

In the control group, 30 larvae of *Cx.quinquefasciatus*were used to measure the activity ofdetoxificationenzymes Inthetemephosgroup,theaverageabsorbancevalueforAChEactivityat0 min was 0.364 ± 0.06 and decreased at 10 min to 0.340 ± 0.009 (Fig. 3). The average absorbancevaluefor GST andoxidase activity for 0 min was 0.325 ± 0.04and 0.176 ± 0.009 andincreasedat 5min to 0.341 ± 0.001 and 0.201 ± 0.003 respectively. The average decrease in AChE activity(Abs/min) from 0 min to 10 minwas 0.002 ± 0.0027 Abs/min.The average increase in GST andoxidase activity from 0 min to 5 min was 0.003 ± 0.001 and 0.005 ±0.001 Abs/min, respectively.Table2showsthatWilcoxonSignedRanksTestresultsfoundthattheactivityofallenzymeswas significantlydifferent(p<0.005).

In the malathion group, the average of AChE activity at 0 min was 0.410 ± 0.006 and decreased at 10 min to 0.357 ± 0.048 (Fig 3). The average GST activity at 0 min was 0.296 ± 0.001 and increased at 5 min to 0.305 ± 0.002. The average of oxidase activity at 0 min was 0.212 ± 0.000 and increased at 5 min was 0.245 ± 0.003. The average decrease in AChE activity (Abs/min) from 0 min to 10 min was 0.002 ± 0.0027 Abs/min. The average increase in GST and oxidase activity from 0 min to 5 min was 0.003 ± 0.001 and 0.005 ±0.001 Abs/min, respectively. Table 3 shows that Wilcoxon Signed Ranks Test results found that the activity of all enzymes was significantly different (p<0.005).

In the cypermethrin group, the average of AChE activity at 0 min was 1.309 ± 0.008 and increased at 10 min to 1.750 ± 0.048. The average GST activity at 0 min was 0.378 ± 0.004 and increased at 5 min to 0.477 ± 0.004. The average of oxidase activity at 0 min was 0.240 ± 0.002 and decreased at 5 min was 0.228 ± 0.003 (Fig 3). The average increase in AChE activity from 0 min to 10 min was 0.044 ± 0.005 Abs/min. The average increase in GST activity from 0 min to 5 min was 0.020 ± 0.001. In contrast, the average decrease in oxidase activity from 0 min to 5 min was 0.002 ± 0.003 Abs/min. Table 3 shows that Wilcoxon Signed Ranks Test results found that the activity of all enzymes was significantly different (p<0.005).

In the deltamethrin group, the average of AChE activity at 0 min was 0.619 ± 0.131 and increased at 10 min to 0.900 ± 0.135. The average GST activity at 0 min was 0.299 ± 0.003 and increased at 5 min to 0.309 ± 0.001. The average of oxidase activity at 0 min was 0.216 ± 0.001 and decreased at 5 min was 0.208 ± 0.006 (Fig 3). The average increase in AChE activity from 0 min to 10 min was 0.028 ± 0.013 Abs/min. The average increase in GST activity from 0 min to 5 min was 0.002 ± 0.001. In contrast, the average decrease in oxidase activity from 0 min to 5min was 0.002 ± 0.001 Abs/min. Table 3 shows that Wilcoxon Signed Ranks Test results found that the activity of all enzymes was significantly different (p<0.005).

## *The histopatological midgut cells of Cx.quiqnuefasciatus larvae*

Table 4 shows the toxicity of insecticides in destroying midgut epithelial cells of *Cx. quinquefasciatus*larvae. A total of 40 *Cx. quinquefasciatus*larvaeweretestedtomeasurethetoxicity of insecticides; temephos (n = 10),malathion (n = 10),cypermethrin (n = 10), anddeltamethrin (n = 10).All insecticides caused damageto epithelial cells (EC). Microvilli (Mv),peritrophicmembrane(PM),andfoodbolus(FB)wereslightlydamagedbyallinsecticides.Therangeof damage to EC was 90%-100%, Mv was 30%-50%, PM was 10%- 20%, dan FB was only 10%.Additionally, the Kruskal-Wallistest showed there were significant differences in damage to EC, Mv,PM,andFBin alltestedinsecticides(p <0.05).

The histopathological midgut changes of the *Cx.quinquefasciatus*larvae were examined by light microscopy. The following midgut sections were investigated: FB, PM, Mv, and EC. Overall, the midgut of the healthy *Cx.quinquefascaitus*used as the control group was normal. FB, PM, Ep, Mv, and EC allexhibited normal structures. The epithelial layer consisted of the single-layered epithelium and columnar,goblet, and degenerative cells, and it was distributed throughout the anterior and posterior regions of themidgut.TheEpithelial layerwaslimited internallybyafinePMandprovidedbyMv(Fig.4Aand4B).

Intheinsecticide-treatedlarvae,temephos,malathion,cypermethrin,anddeltamethrininducedserious damages to FB, PM, EC, and Mv (Fig 5). Damage to EC included morphological deformities of cellsto be small orshrink so that the shapeof these cellswasnotnormal.Damage to Mv includeddeformity of microvilli morphology to become irregular.Damage to the PMshows the membrane tobe damaged or destroyed. Damageto FBshowsbrokenordestroyed FB.

This study focused on the ultrastructural EC of Ep in the midgut of *Cx.quinquefasciatus*larvae induced by malathion and deltamethrin. Malathion and deltamethrin were used as representatives of the organophosphate and pyrethroid groups. As seen in Fig 6A and 6B the ECs of the Ep in the larval midgut were damaged by malathion and deltamethrin respectively. The treatment of malathion and deltamethrin showed that the Mv, nuclei (N,) and nucleoli in the ECs ruptured so that EC structures such as cilia, lysosome, centriole, microtubule, Golgi apparatus, nuclear membrane, mitochondrion, cell membrane, cytoplasm, ribosome, and endoplasmic reticulum were rupture and disappeared (Fig 6A and 6B). The findings demonstrated that malathion and deltamethrin destroyed the EC.

## Discussion

The present study found that *Cx.quinquefasciatus* larvae were completely susceptible to temephos and deltamethrin, while *Cx.quinquefasciatus* larvae to malathion and cypermethrin were categorized as moderate susceptibility in Jakarta. It describes the mechanism of susceptibility of *Cx.quinquefasciatus* larvae to insecticides through the activity of detoxifying enzymes and cell cytotoxicity in the digestive system. To the best of our knowledge, in Jakarta, Indonesia, this study is the first to report the susceptibility of *Cx.quinquefasciatus* to OP and PYR in Jakarta and its mechanisms. Thus, temephos and deltamethrin are still effective for controlling *Cx.quinquefasciatus* mosquito population in Jakarta.

In this study, we only used the larval stage of *Cx.quinquefasciatus* to determine the susceptibility status of *Cx. quinquefasciatus* larvae to temephos, malathion, cypermethrin, and deltamethrin in Jakarta. This study correlates with previous studies that only used the larval stage of *Cx.quinquefasciatus*. For example, DeLisi et al. [35] in East Baton Parish, Louisiana, only used *Cx.quinquefasciatus* larvae to assess the susceptibility of *Cx.quinquefasciatus* to larval insecticides (*Lysinibacillus sphaericus*, spinosad, and temephos). DeLisi's research results found that *Cx. quinquefasciatus* larvae were resistant to temephos due to the frequent use of insecticides to control mosquitoes. Similarly, Boyer et al. [36] only used a laboratory larval strain of *Ae.aegypti* to measure larval tolerance/resistance to temephos, *Bacillus thuringensis var israelensis* (Bti), and toxic vegetable leaf litter. The research on *Cx.quinquefasciatus* larvae and other mosquito species is very useful for detecting the presence of insecticide-resistant or susceptible so that they can design better strategies and help in mosquito control [5, 37,38].

In the present study, in Jakarta, the larvae of *Cx. quinquefasciatus* has been completely susceptible to temephos and deltamethrin because they are rarely used to control *Cx.quinquefasciatus* mosquito. In Jakarta and other big cities in Indonesia, *Cx.quinquefasciatus* mosquitoes are not prioritized in mosquito control programs. However, *Ae.aegypti* mosquito, as a vector of DHF, is a priority to control because cases of DHF that cause death in humans are often found every year in Indonesia [31,39]. In Jakarta, the use of insecticides for the control of *Ae.aegypti*, namely cypermethrin (pyrethroid group), is in accordance with the regional regulations of the Jakarta government [40]. Temephos, malathion, and deltamethrin were not used to control *Ae.aegypti* mosquito. Thus, *Cx.quinquefasciatus* larvae are completely susceptible to temephos and deltamethrin need to be monitored by health workers [41,42] and Jakarta Government officials.

Thongwhat and Bunchu [43] reported that *Ae.aegypti* larvae were susceptible to temephos in Muang District, Phitsanulok Province, Thailand because of the infrequent use of temephos. In a study in Malaysia, Low et al [44] reported that *Cx.quinquefasciatus* larvae were resistant to malathion because of the over-usage of organophosphorus insecticides. However, one of the studied sites, Trengganu, Malaysia, reported that *Cx.quinquefasciatus* larvae were susceptible to DDT, propoxur, malathion, and permethrin. In addition, *Cx.quinquefasciatus* larvae were most susceptible to permethrin than DDT, propoxur, and malathion due to their excessive dependence on these pyrethroid-based household insecticide products such as d-allethrin,d- trans allethrin, transfluthrin, prallethrin, s-biollethrin, deltamethrin, d-henothrin, and permethrin, resulting in low resistance to permethrin [44].

In Jakarta, the susceptibility of *Cx.quinquefasciatus* larvae to temephos and malathion was associated with a significant reduction in AChE activity (Table 3). Temephos and malathion belong to the class of OP that has AChE targets [8,45]. The significant decrease in AChE activity was a mechanism for the susceptibility of *Cx.quinquefasciatus* larvae to temephos and malathion. The findings of this study are in accordance with a study in Western Venezuela where it was reported that *Ae.aegypti* larvae were susceptible to temephos without overexpression of enzymes such as AChE [46]. However, temephos and malathion resistance in the field *Ae.albopictus* in Malaysia was associated with the insensitive AChE activity [47]. Similarly, in Malaysia, malathion resistance in fields *Cx.quinquefasciatus* was associated with the insensitive AChE activity [48].

Cypermethrin and deltamethrin belonged to the PYR group which are toxic to the axon. PYR affects nerve fibers associated with the opening and closing of the VGSC [49]. PYR binds gate, it results in continuous nerve stimulation and tremor in poisoned organisms, resulting in uncoordinated movements [50] and altered behavior patterns such as muscle fasciculations. Seizure, and death [12,51]. Thus, cypermethrin and deltamethrin killed larvae of *Cx.quinquefasciatus* through the blocking gate of VGSC because they had on target VGSC. However, many studies reported that the gene of VGSC is the target site of PYR, and mutations in this gene cause knockdown resistance (*kdr)* [52]. The present study did not detect mutations of the VGSC gene due to deltamethrin killing 100% *Cx.quinquefasciatus* larvae in five sites in Jakarta. However, in Jakarta, permethrin (PYR group) killed 40 % of female *Ae.aegypti* and found V1016G gene mutation with resistance phenotypes to 0.75% permethrin [53].

*Cx.quinquefasciatus* larvae that were completely susceptible to deltamethrin showed a significant decrease in oxidase activity. Likewise, *Cx.quinuqefasciatus* larvae exposed to cypermethrin showed a significantly decreased oxidase activity. Research on oxidase activity was aimed to explain the mechanism of resistance or susceptibility of the larval or adult stages of mosquitoes to all classes of insecticides [23]. The results of this study were the complete susceptibility of *Cx quinquefasciatus* larvae to deltamethrin associated with a significant reduction of oxidase activity (Table 3). However, another study reported that PYR resistance in field *Cx.quinquefasciatus* in Collier County, Florida, was associated with oxidase and esterase activity [54]. Thus, a significant decrease in oxidase activity is a mechanism for the susceptibility of *Cx.quinquefasciatus* larvae to PYR classes such as deltamethrin and cypermethrin.

The findings of this study showed that all tested insecticides exhibited more damage to the midgut ECs than other parts of the midgut. Statistically, there were significant differences in midgut ECs damage with other parts of the midgut in all tested insecticides (Table 4). The results of TEM observations showed that there was damage to midgut ECs in the form of damage to cell membranes, mitochondria, nucleolus membranes, and other cell organelles (Fig. 6A and 6B). The effect of temephos, malathion, cypermethrin, and deltamethrin on the damage of the larval midgut ECs in *Cx.quinquefasciatus* has not been reported. These findings are in accordance with Lu et al [15] who reported that deltamethrin causes oxidative stress resulting in an increase in ROS. The increase in ROS results in cell damage. Richardson et al [55] reported that permethrin (PYR group) might have a variety of toxic effects on animals and humans alike, such as neurotoxicity, immunotoxicity, cardiotoxicity, hepatotoxicity, reproductive, genotoxic, and hematologic effects, digestive system toxicity, and cytotoxicity. The present study demonstrated that temephos, malathion, cypermethrin, and deltamethrin exhibited digestive system cytotoxic activity with evidence of the midgut cell damage of *Cx.quinquefasciatus* larvae.

Chargui et al [56] reported histopathological alterations of the liver and kidney of female rats exposed to low doses of deltamethrin (at doses of 0.003, 0.03, and 0.3 mg/kg bw/d) after 30, 45, and 60 d, respectively. Deltamethrin induces apoptosis in SH-SY5Y cells and reduces PINK1 expression in human neuroblastoma cells [57]. Tubefenozid, a novel nonsteroidal ecdysone agonist, showed that it caused serious damage in the midgut of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) larvae [58]. These cells show a damaged striated border with the release of protrusions to the midgut lumen, damaged nuclear membrane, and nucleus with condensed chromatin, and an increase in the number of autophagic vacuoles. Mitochondria were modified into nano tunnels which might be a piece of evidence that tebufenozide induces damage to cells, resulting in cell death, proved by immunofluorescence analyses [58].

The study toxic effect of OP and PYR on insects, animals, and humans is very important because insecticides are used worldwide [55]. The present study demonstrated that the toxic effects of temephos, malathion, cypermethrin, and deltamethrin on the digestive system of *Cx.quinquefasciatus* larvae are the same as in rats [56]. In Jakarta, the various toxic effects of insecticides on animals and humans have not been studied so far. Thus, in the future, research is needed on the toxic effects of insecticides on animals and humans. This study is useful for providing data on various toxic effects of insecticides on animals and humans so as help to improve and evaluate mosquito control programs.

## Conclusions

In five sites of Jakarta, *Cx quinquefasciatus* larvae are completely susceptible to temephos and deltamethrin, but moderately susceptible to malathion and cypermethrin. Metabolic enzymes and the midgut cell cytotoxicity are mechanisms of susceptibility of *Cx.quinquefasciatus* larvae to insecticides. This study provides baseline data to improve mosquito control programs.

## Declarations

# Author's Contribution

RS: designed the study, RS, APA and RRF: collected samples from the field and conducted the larval bioassay. RS, RRF, NSL and LS: conducted the histopatological examination of the midgut, RS and RRF: conducted TEM, RS APA, Yul and Fat: conducted the biochemical assay, RS, APA, and RRF: analyzed the data. RS, APA, RRF, LS, FAT, Yul, NSL: drafted the manuscript, all the authors revised, read, and approved the final manuscript.

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# Competing Interest

The authors declare that they have no competing interest

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## Tables

Table 1

Description of VBD cases, mosquito control, usage of insecticides, household insecticides, electric mosquito trap, and natural breeding place in Jakarta.

| No | Description | DKI Jakarta | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 1 | **VBD cases (in 2019)** | | | | | |
| | Malaria | 27 | 2 | 5 | 7 | 11 |
| | DHF | 3014 | 491 | 922 | 2305 | 1975 |
| | Filariasis | 17 | 4 | 0 | 2 | 0 |
| 2 | **Mosquito control** | | | | | |
| | Anopheles sp | No | No | No | No | No |
| | Aedes sp | Active | Active | Active | Active | Active |
| | *Cx.quinquefasciatus* | No | No | No | No | No |
| 3 | **Usage of Insecticides** | | | | | |
| | Pyrethroids | Active | Active | Active | Active | Active |
| | Organocholine | No | No | No | No | No |
| | Organophosphate | No | No | No | No | No |
| | Carbamate | No | No | No | No | No |
| 4 | **Natural breeding place** | | | | | |
| | Anopheles sp | - | - | + | - | - |
| | Aedes sp | + | + | + | + | + |
| | *Cx.quinquefasciatus* | + | + | + | + | + |

Note: 1 = East, 2= central, 3= north, 4= west, 5= south,

natural breeding places + = available, - = not available

Table 2

Mortality rate of *Cx.quinquefasciatus* larvae after exposed by insecticides

| Insecticides | Concent. | Locations for collecting *Cx.quinquefasciatus* larvae | | | | |
|---|---|---|---|---|---|---|
| | (ppm) | 1 | 2 | 3 | 4 | 5 |
| Temephos | 1.25 | 100% (125/125) | 100% (125/125) | 100% (125/125) | 100% (125/125) | 100% (125/125) |
| | 6.25 | 100% (125/125) | 100% (125/125) | 100% (125/125) | 100% (125/125) | 100% (125/125) |
| | 31.25 | 100% (125/125) | 100% (125/125) | 100% (125/125) | 100% (125/125) | 100% (125/125) |
| | 156.25 | 100% (125/125) | 100% (125/125) | 100% (125/125) | 100% (125/125) | 100% (125/125) |
| Malathion | 1.0 | 100% (125/125) | 98.4% (123/125) | 99.2% (124/125) | 96.8% (121/125) | 99.2% (124/125) |
| Cypermethrin | 0.75 | 99.2% (124/125) | 99.2% (124/125) | 100% (125/125) | 98.4% (123/125) | 90.4% (122/125) |
| Deltamethrin | 0.25 | 100% (125/125) | 100% (125/125) | 100% (125/125) | 100% (125/125) | 97.6% (125/125) |

Note: 1 = East, 2= central, 3= north, 4= west, 5= south,

Table 3

Detoxification enzyme activity of *Cx.quinquefasciatus* larvae after exposed by insecticides

| Insecticides | N | Enzyme | Mean of Absorbance | | Activity | Wilcoxon Test | |
|---|---|---|---|---|---|---|---|
| | | | T0 | T5 0r T10 | | Z | Sig. (2-Tailed) |
| Temephos | 50 | AChE | 0.364 ± 0.006 | 0.340 ± 0.026 | Decrease | -8.690 | 0.000 |
| | | GST | 0.325 ± 0.004 | 0.341 ± 0.001 | Increase | -8.815 | 0.000 |
| | | oxidase | 0.176 ± 0.009 | 0.201 ± 0.003 | Increase | -8.713 | 0.000 |
| | Malathion | 50 AChE | 0.410 ± 0.006 | 0.357 ± 0.048 | Decrease | -8.690 | 0.000 |
| | | GST | 0.296 ± 0.001 | 0.305 ± 0.002 | Increase | -8.745 | 0.000 |
| | | oxidase | 0.212 ± 0.003 | 0.245 ± 0.003 | Increase | -8.701 | 0.000 |
| Cypermethrin | 50 | AChE | 1.309 ± 0.008 | 1.750 ± 0.048 | Increase | -2.738 | 0.006 |
| | | GST | 0.378 ± 0.004 | 0.477 ± 0.004 | Increase | -8.718 | 0.000 |
| | | oxidase | 0.240 ± 0.002 | 0.228 ± 0.014 | Decrease | -8.701 | 0.000 |
| Deltamethrin | 50 | AChE | 0.619 ± 0.131 | 0.900 ± 0.135 | Increase | -8.697 | 0.000 |
| | | GST | 0.299 ± 0.003 | 0.309 ± 0.001 | Increase | -8.713 | 0.000 |
| | | oxidase | 0.216 ± 0.001 | 0.208 ± 0.006 | Decrease | -8.719 | 0.000 |

Table 4

Cytotoxic activity of insecticides in destroying midgut epithelial cells of *Cx.quinquefasciatus* larvae.

| Insecticide | n | EC | Microvilli | PM | FB | Kruskal-WallisTest | |
|---|---|---|---|---|---|---|---|
| | | | | | | Chi- square | Sig |
| Temephos | 10 | 100% (10/10) | 40% (4/10) | 20% (2/10) | 10% (1/10) | 65.081 | 0.000 |
| Malathion | 10 | 90% (9/10) | 30% (3/10) | 20% (2/10) | 10% (1/10) | | |
| Cypermetrhin | 10 | 100% (10/10) | 40% (4/10) | 10% (1/10) | 10% (1/10) | | |
| Deltamethrin | 10 | 100% (10/10) | 50% (5/10) | 20% (2/10) | 10% (1/10) | | |

EC= epithelial cell, PM = periotrophic membrane, FB= food bolus

## Figures

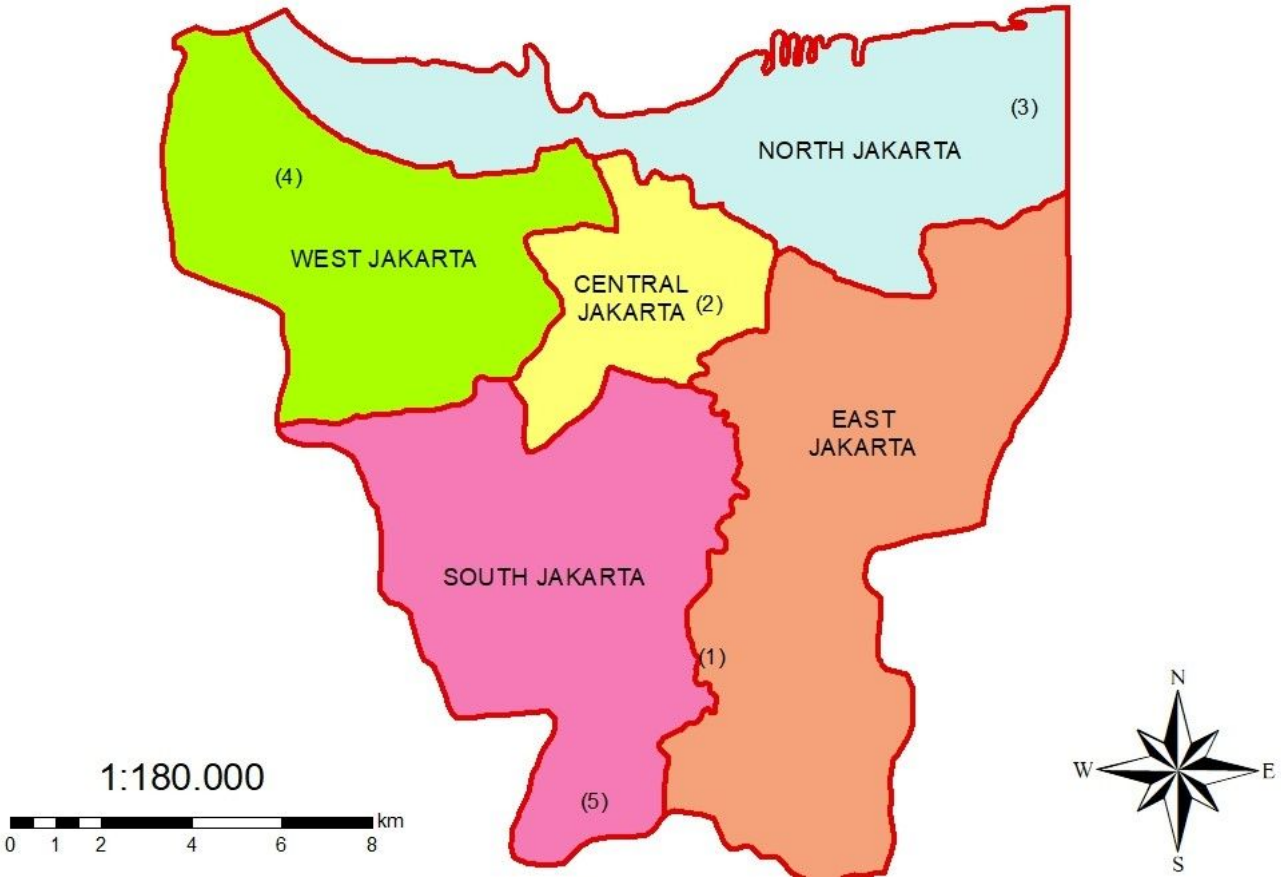


**Figure 1**

Collection sites of *Cx.quinquefasciatus* larvae in Jakarta. Ujung Gedong, East Jakarta (1), Johar Baru, Central Jakarta (2), Marunda North Jakarta (3), Cengkareng, West Jakarta (4), and Situ Babakan, South Jakarta (5).

**Figure 2**

Representation of a breeding place in East Jakarta

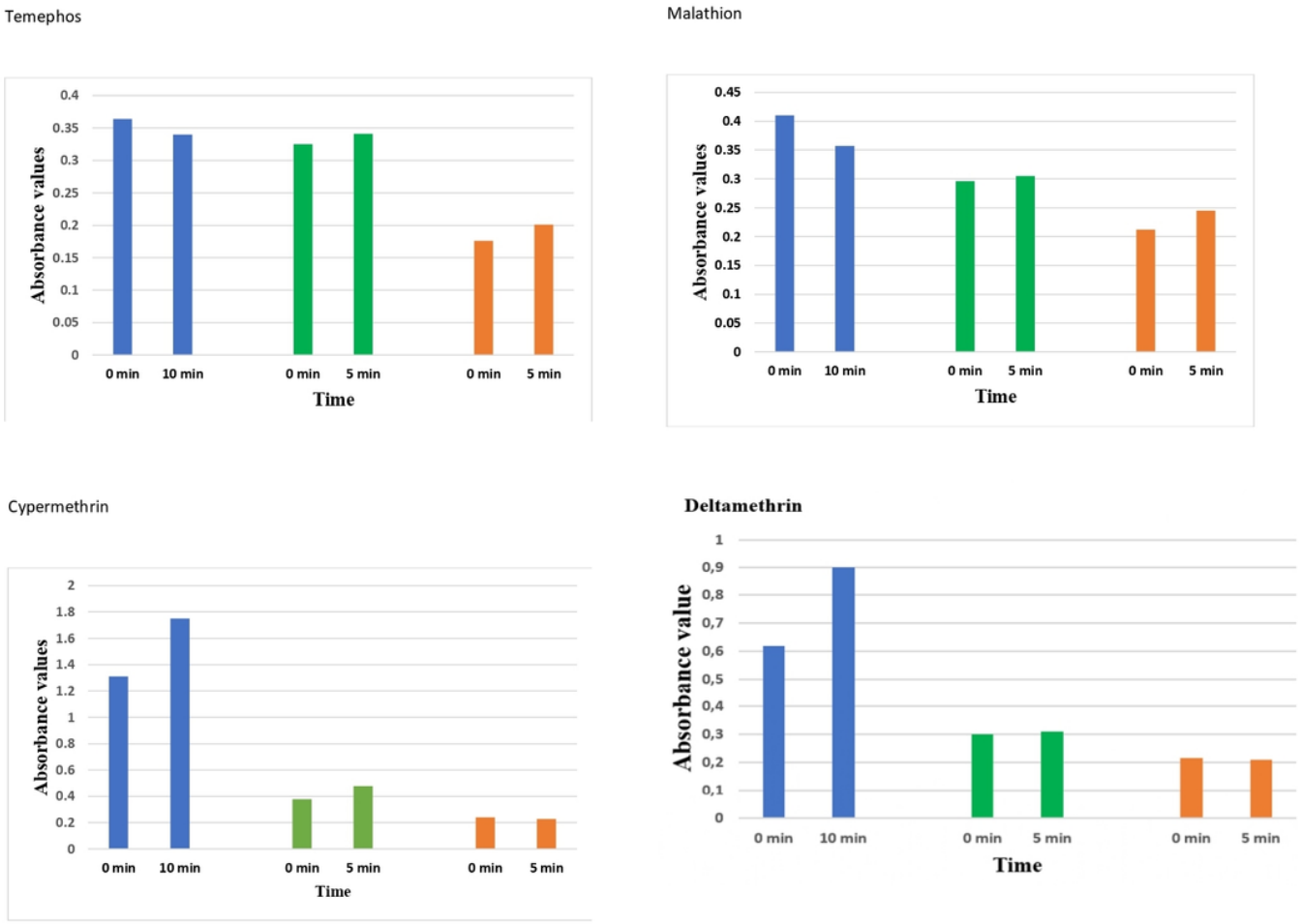


Figure 3

**3A and 3B**. Activity of AChE, GST, and oxidase of *Cx.quinquefasciatus* larvae

after exposed by insecticides. A= temephos and malathion, B= cypermethrin and deltamethrin.

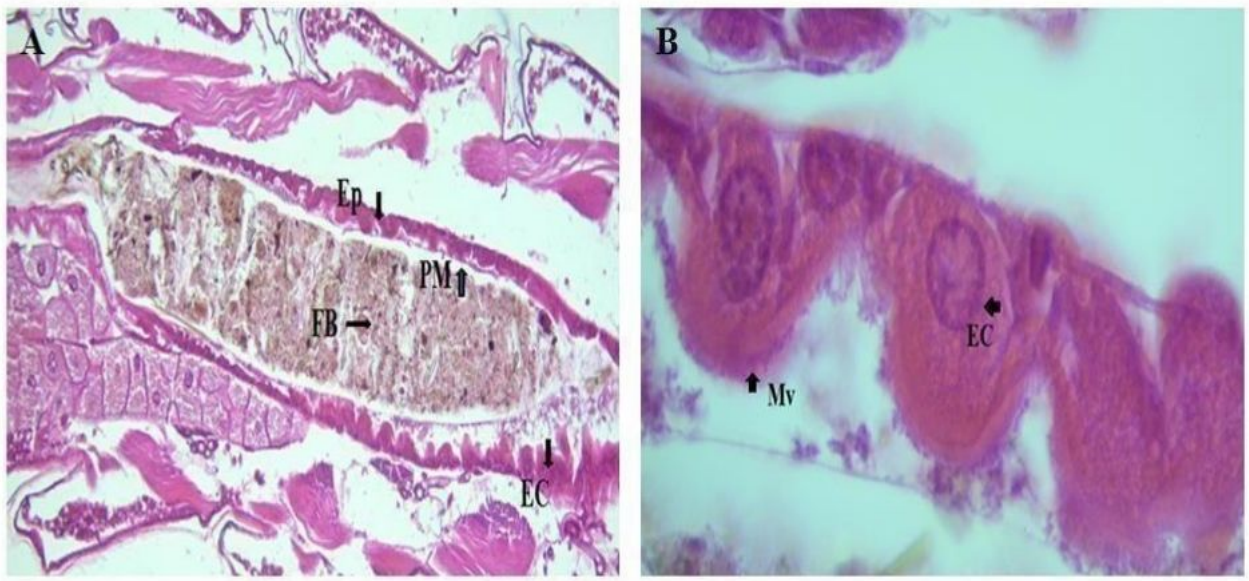


Figure 4

**4A and 4B**. The midgut of a healthy *Culex quinquefasciatus* larva stained with H&E. A = 10x magnification, B = 100x magnification. FB = food bolus, PM = peritrophic membrane, Ep = epithelial layer, EC = epithelial cell, Mv = microvilli

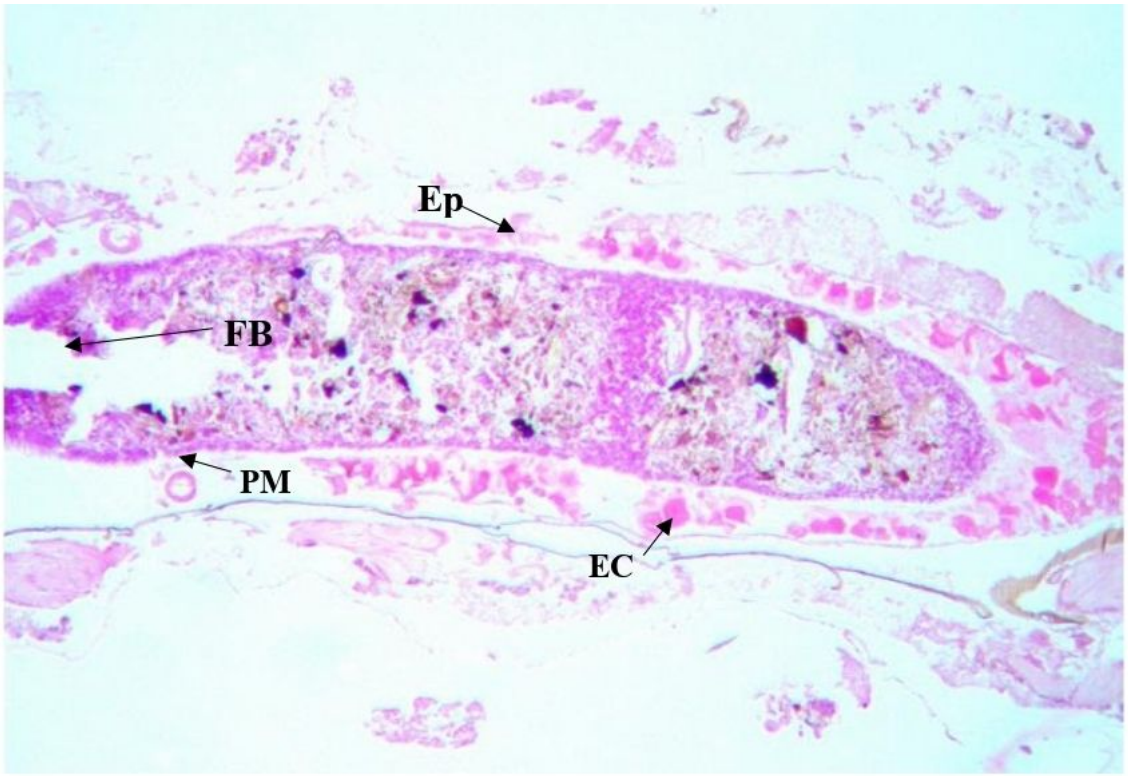


**Figure 5**

Representation of the histopatological midgut damage of *Culex quinquefasciatus* larvae after being treated by insecticide, stained with H&E, 10x magnification. FB = food bolus, Ep = epithelial layer, EC = epithelial cell.

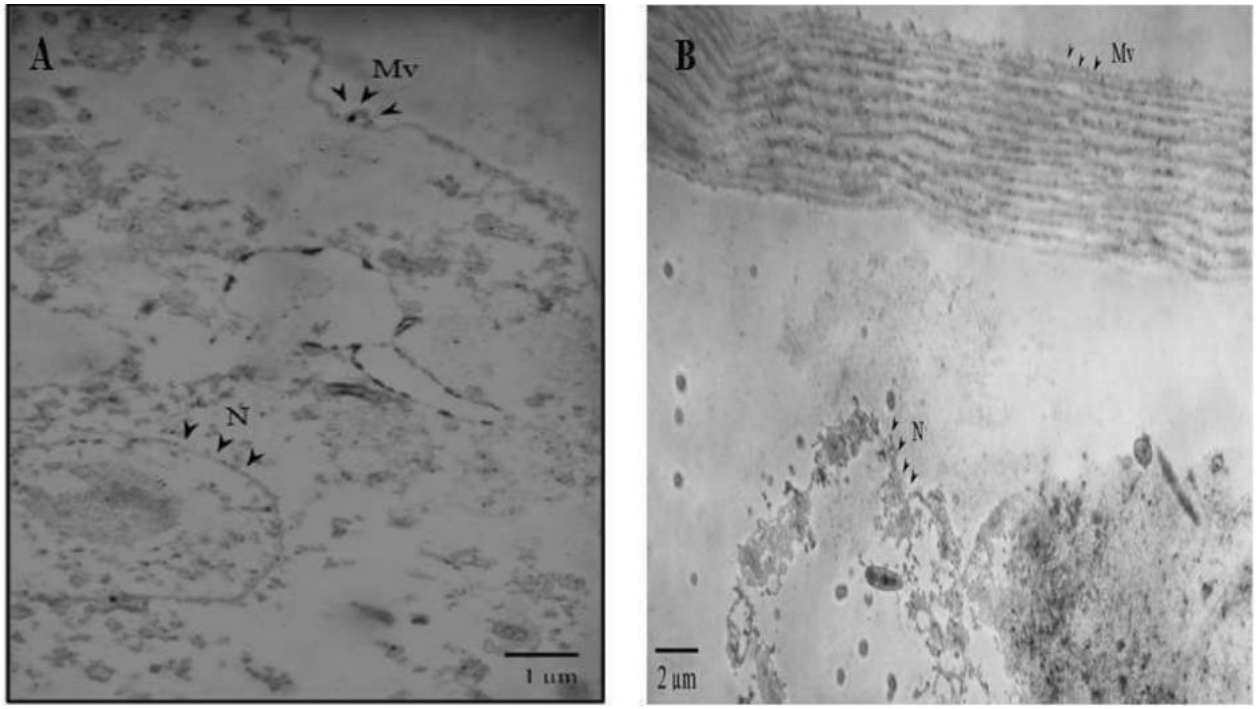


**Figure 6**

**6A and 6B**. Ultrastructural ECs in the Ep of the *Culex quinquefasciatus* midgut larvae induced by malathion (7A) and deltamethrin (7B). Transmission electron microscopy (TEM), N= nucleus, Mv = microvilli.