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Rizal Subahar
Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba 6, Jakarta, Indonesia

Lia Savitri Eka Nur
Medical Doctor Program, Faculty of Medicine, Universitas Indonesia, Jl. Salemba 4, Jakarta 10430, Indonesia

Ayleen Huang
Medical Doctor Program, Faculty of Medicine, Universitas Indonesia, Jl. Salemba 4, Jakarta 10430, Indonesia

Rafaella Shiene Wijaya
Medical Doctor Program, Faculty of Medicine, Universitas Indonesia, Jl. Salemba 4, Jakarta 10430, Indonesia

Yulhasri Yulhasri
Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jln. Salemba Raya 4, Jakarta 10430, Indonesia

Lisawati Susanto
Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba 6, Jakarta 10430, Indonesia

Rawina Winita
Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba 6, Jakarta 10430, Indonesia

Corresponding Author:
Rizal Subahar
Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba 6, Jakarta, Indonesia

Evaluation of pure camphor ($C_{10}H_{16}O$) against wild-caught *Culex quinquefasciatus* larvae in East Jakarta, Indonesia: Detoxification enzymes and histopathological midgut

Rizal Subahar, Lia Savitri Eka Nur, Ayleen Huang, Rafaella Shiene Wijaya, Yulhasri Yulhasri, Lisawati Susanto and Rawina Winita

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Abstract
Filariasis, transmitted by the lymphatic filariasis vector *Culex quinquefasciatus*, is still a global public health issue. Resistance to *Cx. quinquefasciatus* is caused by the eradication of filariasis with synthetic insecticides. The aim of the study was to evaluate the toxicity of pure camphor on *Cx. quinquefasciatus*, with a focus on detoxifying enzymes and midgut histopathological abnormalities. *Cx. quinquefasciatus* larvae are field-collected wild-strain larvae. The WHO protocol was followed for the larval bioassays. Pure camphor concentrations of 0.5, 1.5, 10.5, 25.5, and 50 ppm were applied to larvae for 24, 48, and 72 h, with 5 replications. The biochemical method was used to test detoxification enzymes such as acetylcholinesterase (AChE), glutathione S-transferase (GST), and oxidase. The routine histopathological examination was performed on the larvae's histopathological midguts. At 48 h, pure camphor caused 100% mortality of *Cx. quinquefasciatus* larvae. LC_{50} and LC_{90} values were found to be 2.32 and 12.40 ppm respectively. AChE and oxidase activity were both significantly lower (p<0.05). Damage to the food bolus (FB) and peritrophic membrane (PM), broken epithelial layers (EP), changes in the size and shape of epithelial cells (EC), and microvilli (Mv) damage were all observed in larval midguts. In our study, pure camphor demonstrated larvicidal activity via decreased AChE and oxidase activity, as well as midgut damage.

Keywords: *Culex quinquefasciatus*, pure camphor, midgut histopathology, detoxification enzymes

1. Introduction
The southern house mosquito or *Culex quinquefasciatus* is one of the vectors that transmit mosquito-borne disease (MBD). MBDs transmitted by *Cx. quinquefasciatus* include Rift Valley fever virus, Japanese encephalitis, St. Louis encephalitis, West Nile virus, Bancrofti lymphatic filariasis, Zika virus, and Equine encephalitis virus [1-3]. There are 1.3 billion people worldwide who are at risk of contracting filariasis [4, 5]. World Health Organization (WHO) estimates approximately 859 million people in fifty countries are still at risk of lymphatic filariasis, and preventive chemotherapy is needed to stop the spread of this parasitic infection [5]. According to the Ministry of Health of the Republic of Indonesia, there were 9,906 cases of filariasis in Indonesia in 2020, distributed across 34 provinces, with the highest cases of filariasis in Indonesia were in the eastern part of the country, Papua with 3,615 cases and East Nusa Tenggara with 1,534 cases [6].

Vaccines for Japanese encephalitis, Bancrofti filariasis, and Zika infections have yet to be discovered. Thus, to eradicate the disease, synthetic insecticides are still used to control *Cx. quinquefasciatus* [7]. However, the long-term use of these insecticides causes air pollution, disrupts human health, and causes mosquito resistance to insecticides [8, 9]. Insecticide resistance in *Cx. quinquefasciatus* has been reported in several countries, including Brazil [2], Mississippi [10], Saudi Arabia [11], Sri Lanka [12], Guadelaupe [13] and Thailand [14].

It is critical to use alternative insecticides made from natural plant ingredients to combat the problem of insecticide resistance because these natural ingredients are not toxic to humans or other animals and do not cause mosquito resistance [15].

Camphor ($C_{10}H_{16}O$, monoterpene) is a volatile, aromatic, crystalline monoterpene ketone derived from *Cinnamomum camphora* wood or synthesized from turpentine [16]. Camphor also had insecticidal anti-inflammatory, antimicrobial, analgesic, anticonvulsant, antiviral, antituberculosis, anticancer, and antioxidant properties [16-18]. Comphor compounds are essential oil compounds found in plants such as *Cinnamomum camphora* [19], *Rosamary officinalis* [20], and others. Camphor-containing crude extracts of these plants have been shown to possess larvicidal activity against mosquito species such as Aedes spp. [21], Anopheles spp [22]. Culex spp [23]. Previously conducted research on camphor's larvicidal activity against mosquito larvae did not explain how camphor kills mosquito larvae.

Until now, there has been no research on pure camphor compounds to test larvicidal activity against *Cx. quinquefasciatus* and its mechanism, particularly in Indonesia. We hypothesized that pure camphor kills *Cx. quinquefasciatus* larvae by causing changes in midgut histology and detoxification enzyme activity. To prove this hypothesis, we studied the activity of *Cx. quinquefasciatus* larvae obtained from the field (wild-caught *Cx. quinquefasciatus* larvae) using pure camphor. The study concentrated on the histopathological midgut of *Cx. quinquefasciatus* larvae because the midgut, particularly the epithelial cells (EC), peritrophic membrane (PM), food bolus (FB), epithelial layer (EP), and microvilli (Mv), are extremely sensitive to bioactive compounds [24, 25].

Any changes in the midgut of mosquito larvae or other insects can alter detoxifying enzyme activity [26]. These detoxifying enzymes are essential for removing toxins from the bodies of mosquito larvae and other insects [27]. Susceptible mosquitoes or other insects have reduced detoxification enzyme activity, whereas resistant mosquitoes or other insects have increased detoxification enzyme activity [28]. This study, it was investigated whether pure camphor compounds could increase or decrease detoxifying enzymes' activity including acetylcholinesterase (AChE), glutathione S-transferase (GST) and oxidase.

Jakarta was chosen as the location of this study because upon evaluation, 22 cases of chronic filariasis are extant with one new case [29]. There are still many of natural breeding grounds for *Cx. quinquefasciatus*, such as sewers in Jakarta's housing complexes. In Jakarta, there are also many ornamental fish sellers who sell *Cx. quinquefasciatus* larvae as food for ornamental fish and many Jakarta residents buy these larvae, so there is a risk of more *Cx. quinquefasciatus* larvae spreading. In addition, larval control of *Cx. quinquefasciatus* has not been made a top priority in Mosquito Control Program of Jakarta and some other cities in Indonesia.[29] The study was conducted to evaluate the toxicity of pure camphor compound on wild-caught *Cx. quinquefasciatus* larvae with a particular emphasis on midgut histopathological abnormalities and detoxifying enzymes.

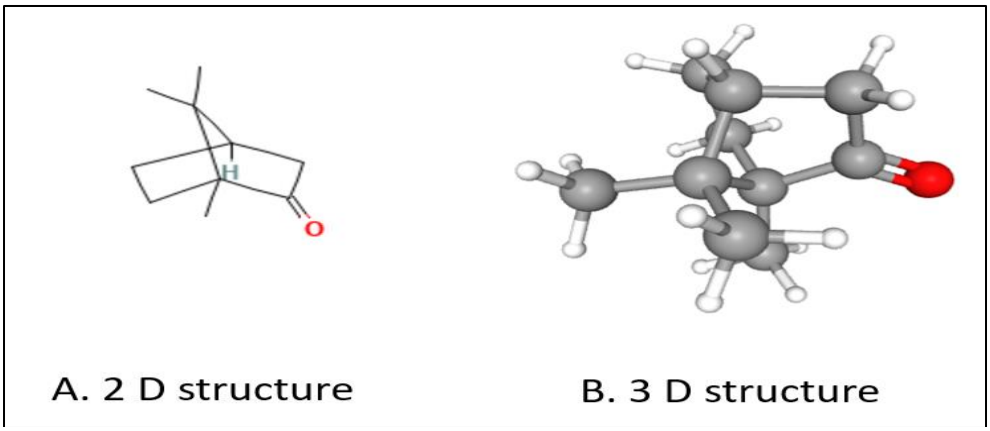
2. Materials and Methods

2.1 Ethical statement

The study has received ethical approval from Universitas Indonesia's Faculty of Medicine (No. KET-007/UN2.F1.D1.2/PDP01./Riset-2/2022).

2.2 Camphor

Commercial camphor (pure compound, $C_{10}H_{16}O$,) was purchased from an Indonesian manufacturer (Fig. 1). Camphor stock solution was made through dissolving 50 mg of camphor in 500 ml of distilled water. Camphor concentrations of 0,5, 1,5, 10,5, 25,5, and 50 ppm were prepared from the stock solution.



Source: https://pubchem.ncbi.nlm.nih.gov/compound/2537

Fig 1: Structure of camphor.

2.3 Larval Bioassay

A larval bioassay was performed using WHO standards [30]. Wild-caught *Cx. quinquefasciatus* larvae (3^{rd} to 4^{th} instars) were used in the bioassay. In a 300 mL paper cup, 25 larvae were placed in 200 mL of camphor at concentrations of 0.5, 1.5, 10.5, 25.5, and 50 ppm. The larval bioassay was replicated 5 times. In the control group, 25 *Cx. quinquefasciatus* larvae were placed in a 200 mL paper cup containing tap water. The larval bioassay was performed after 24, 48, and 72 h.

2.4 Biochemical assays

In the current study, 60 *Cx. quinquefasciatus* larvae were used to examine detoxifying enzyme activity, with 10 larvae from the control, 50 from camphore; for each camphor concentration (0.5, 1.5, 10.5, 25.5, and 50 ppm), 10 dead larvae of *Cx. quinquefasciatus* were tested for enzyme activity. To check the activity of detoxification enzymes, the five larvae from each concentration were made into one or pool. The activities of AChE, GST, and oxidase were

investigated as part of the detoxifying enzyme analysis.

2.4.1 AChE assay
As previously described, enzyme activity assays were used to assess AChE activity [28]. Dead *Cx. quinquefasciatus* larvae were collected and then homogenized in 1 mL of 0.25 M KPO4 (pH 7.2). Aliquots of the test sample homogenates with the volume of 100 µL were loaded into triplicate ELISA microplate wells at room temperature. Similarly, 100 µL aliquots of the control group were added to three microplate wells in triplicate. Acetylcholine iodide (ACTH) and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were added to each well (100 µL of each), then absorbance was measured immediately (T_0) and again after 10 minutes (T_{10}) with an ELISA reader at 414 nm. Absorbance per minute, or Abs/min, was the unit of AChE activity. The ELISA reader was made in Finland by Thermo Fisher Scientific™ cat # 51119000.

2.4.2 GST assay
As previously described, the enzyme activity assays were used to analyze GST activity [28]. Dead *Cx.quinquefasciatus* larvae were collected and then homogenized in 1.0 mL 0.25 M KPO_4 (pH 7.2). Aliquots of each homogenate with the volume of 100 µL were loaded into triplicate ELISA microplate wells at room temperature; 100 µL of the control group aliquots were prepared similarly. Aliquots (100 µL) of reduced glutathione solution (Sigma G4251) and 1-chloro-2,4'- dinitrobenzene (cDNB) were added, and the plates were read immediately (T_0) with an ELISA reader at 340 nm and again at 5 min (T_5). The unit of GST activity was absorbance per minute, or Abs/min.

2.4.3 Oxidase assay
As previously described, enzyme activity assays were used to assess oxidase activity [28]. The dead *Cx. quinquefasciatus* larvae were collected from larval bioassays and homogenized in 1000 L 0.25 M KPO4 solution (pH 7.2). Positive controls included the following: 1:55 (22 µL stock, 1.2 mL KPO4 buffer) and 1:110 (11 µL cytochrome stock, 1.2 mL KPO4 buffer). 100 µL triplicate aliquots of the test sample homogenates were added in ELISA microplate wells, then 100 L KPO4 was added to the negative and positive control wells. A 100 µL cytochrome-C positive control (cytochrome-C bovine heart) solution was added, followed by a 200 µL TMBZ solution. Each well received one drop of 3% hydrogen peroxide (H_2O_2) and the plate was incubated for 5 minutes. The plates were immediately read (T_0) with an ELISA reader at 620 nm. Oxidase activity was measured using Absorbance per minute or Abs/min.

2.5 Histopathological examination
The previously described the routine histopathological technique was used [31]. Forty-five dead *Cx. quinquefasciatus* larvae were examined in total, with 5 from the control group and 40 from the treatment groups (20 larvae of eugenol and 20 larvae of piperine). The specimens were fixed using 10% formalin and went through dehidration using increased alcohol concentrations (70%, 80%, 90%, 95%, and 100%). Following that, the specimens were immersed in xylene 1, xylene 2, and xylene 3 solutions and were embedded in paraffin blocks. The paraffin blocks were then cut into sections with the thickness of 5 µm using a manual microtome (Model 320, No. 17664, USA) and feather microtome blades (Feather, S35, Japan). The sections were then stained with hematoxylin and eosin (H&E) and then observed under a light microscope and photographed d using a digital microscopic mounted camera (Zeiss Axiocam ERC 5s, Germany).

2.6 Data Analysis
All experimental data were analyzed using SPSS Statistics ver. 26. Differences in mortality between concentrations of camphore were analyzed using a one-way ANOVA test with one factor if the distribution was normal and a non-parametric test otherwise [32]. Probit analysis was used to calculate LC_{50} and LC_{90} values [33]. To determine the difference in AChE, GST, and oxidase activity, a paired T test was performed with the condition that the data had a normal distribution. If the data does not fit the normal distribution criteria, the Wilcoxon non-parametric test will be used instead [32].

3. Results
3.1 Mortality of wild-caught Cx. quinquefasciatus larvae
This study was able to collect 625 *Cx. quinquefasciatus* larvae (III and IV instars) from the field. The mortality rates for *Cx. quinquefasciatus* larvae after exposure to various concentrations of pure camphor compounds are summarized in Table 1. There were no larvae that died in the control group after 24, 48, or 72 h. At a concentration of 50 ppm, camphor caused 100% larval mortality after 48 h. Camphor concentrations of 10.5 and 25.5 ppm, on the other hand, resulted in 100% larval mortality after 72 h. The mortality of *Cx. quinquefasciatus* larvae was significantly different in each camphor concentration, according to a one-way ANOVA statistical analysis (p<0.05). Camphor was found to be toxic to wild-caught *Cx. quinquefasciatus* larvae in this study, with an LC_{50} of 2.32 ppm (95% CI: 1.98 - 2.77) and an LC_{90} of 12.40 ppm (95% CI: 8.03 - 27.52). The Probit model is significant (p<0.05) according to Probit analysis, and the Chi-square test of the model's goodness is also significant (p< .05).

Table 1: Mortality of wild-caught *Cx. quinquefasciatus* larvae after being exposed to a pure camphor.

Treatment	Concentra-tion (ppm)	N	Mortality rate (%)			p value
			24 h	48 h	72 h	
Control	0	25	0	0	0	0,000
Pure Camphor	0,5	125	10,4 (13/125)	42,4 (53/125)	96 (120/125)	
	1,5	125	23,2 (29/125)	58,4 (73/125)	98,4 (123/125)	
	10,5	125	56,8 (71/125)	87.2 (109/125)	100 (125/125)	
	25,5	125	72 (90/125)	89,6 (112/125)	100 (125/125)	
	50	125	82.4 (103/125)	100 (125/125)	100 (125/125)	

p value was obtained from the one way ANOVA test

3.2 Detoxification enzyme activity

The mean absorbance of detoxifying enzymes in wild-caught *Cx. quinquefasciatus* larvae after exposure to pure camphor is summarized in Table 2. The mean absorbance of the larvae's detoxifying enzymes varied with each concentration of pure camphor. After being exposed to pure camphor, AChE and oxidase activity were lower than in the control group. However, GST activity was higher in the camphor group than in the control group.

Table 2: The mean absorbance of AChE, GST, and oxidase of *Cx. quinquefasciatus* larvae after being exposed to pure camphor.

Treatment	n	AChE		GST		Oxidase	
		0 min	10 min	0 min	5 min	0 min	5 min
Control Camphor	10	0.320	0.326	0.533	0.592	0.452	0.562
0.5 ppm	10	0.301	0.303	0.530	0.655	0.247	0.244
1.5 ppm	10	0.320	0.323	0.571	0.649	0.221	0.242
10.5 ppm	10	0.292	0.295	0.496	0.599	0.172	0.161
25.5 ppm	10	0.300	0.305	0.629	0.969	0.266	0.255
50.0 ppm	10	0.313	0.317	0.559	0.644	0.245	0.240

n= number larvae of *Cx. quinquefasciatus*

Furthermore, the Shapiro-Wilk test revealed that the AChE activity data were normally distributed ($p>0.05$), and the paired T-test revealed a significant increase in AChE activity from 0 to 10 min ($p<0.05$). Figure 2 shows that the AChE activity of pure camphor concentrations of 0.5, 1.5, 10.5, 25.5, and 50.0 ppm were 0.0002, 0.0003, 0.0005, and 0.0004 Abs/min, respectively. AChE activity in the control group was 0.0006 Abs/min.

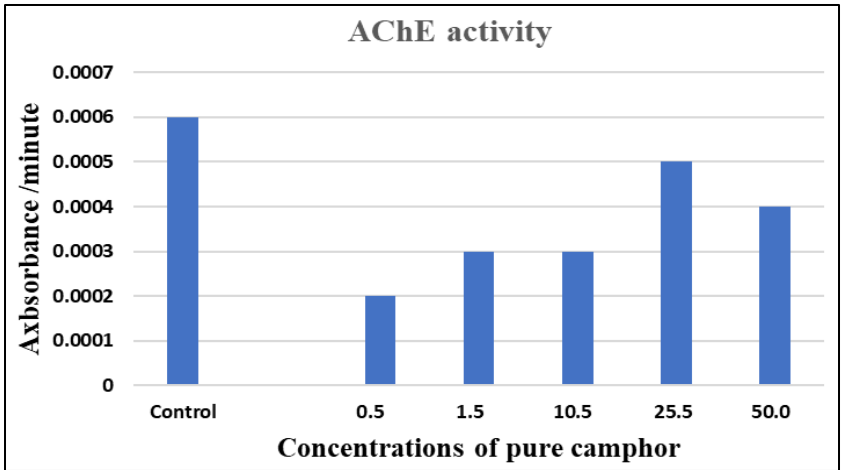


Fig 2: AChE activity of wild-caught *Cx. quinquefasciatus* larvae after being exposed to pure camphor.

GST activity increased in *Cx. quinquefasciatus* larvae exposed to different concentrations of pure camphor from 0 to 5 minutes. The GST activity of pure camphor concentrations of 0.5, 1.5, 10.5, 25.5, and 50.0 ppm was 0.025, 0.0156, 0.0206, 0.0134, and 0.017Abs/min, as shown in Figure 3. The control group's GST activity was 0.0118 Abs/min. Because the GST activity data were not normally distributed, the Wilcoxon test was used, and it revealed that there was a significant increase in GST ($p<0.05$).

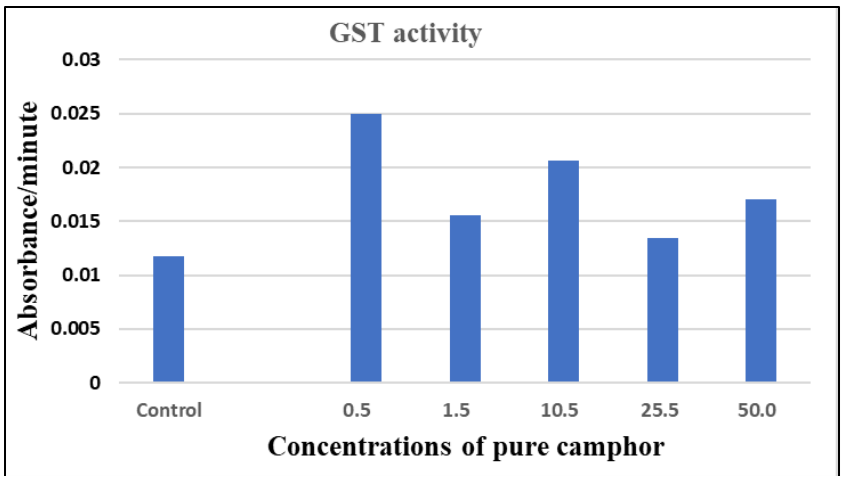


Fig 3: GST activity of wild-caught *Cx. quinquefasciatus* larvae after being exposed to a pure camphor.

Oxidase activity decreased in wild-caught *Cx. quinquefasciatus* larvae exposed to different concentrations of pure camphor (Fig.4). The oxidase activity of pure camphor concentrations of 0.5, 1.5, 10.5, 25.5, and 50.0 ppm was -0.0006, 0.0042, -0.0312, -0.0022, and -0.0010 Abs/min, as shown in Figure 4. The control group's oxidase activity was 0.0220 Abs/min. the Wilcoxon test was used, and it revealed that there was a significant increase in oxidase activity (p<0.05).

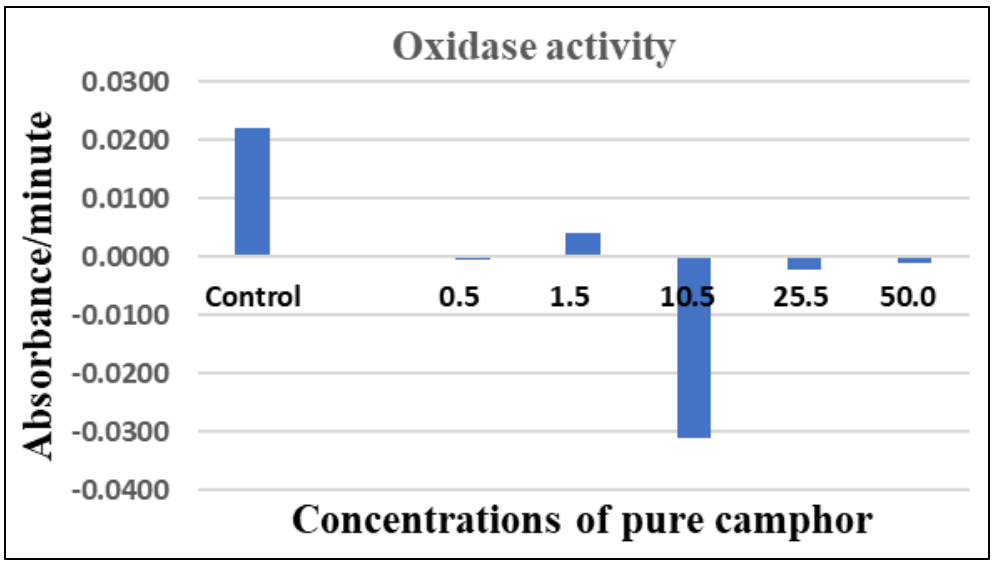


Fig 4: Oxidase activity of wild-caught *Cx. quinquefasciatus* larvae after being exposed to pure camphor.

3.2 Changes in histopathological midgut

For the histopathological examination, 28 larvae of *Cx. quinquefasciatus* were used, one for the control group and 27 for the camphor group. Table 4 summarizes the histopathological results of midgut larvae exposed to various concentrations of camphor. There were no abnormalities found in the larvae's midgut in the control group; the food bolus (FB), peritrophic membrane (PM), epithelial layer (EP), epithelial cells (EC), and microvilli (Mv) all had good or normal structure (Fig 5).

At each concentration, there were abnormalities in the midgut histopathology, in contrast to the camphor group. Overall midgut damage of *Cx. quinquefasciatus* larvae was classified as serious in the camphor group. The larval midgut abnormalities discovered were as follows: 1) the FB was found to be broken or cracked, and there was a narrowing or widening of the FB, 2) the PM was damaged or broken, 3) the EP midgut showed damage, namely the layers were cut off from one another and the EP had an irregular shape, 4) EC is damaged where the cell shape is irregular and the cell size shrinks, and 5) Mv was also damaged to become smaller and irregular in shape.

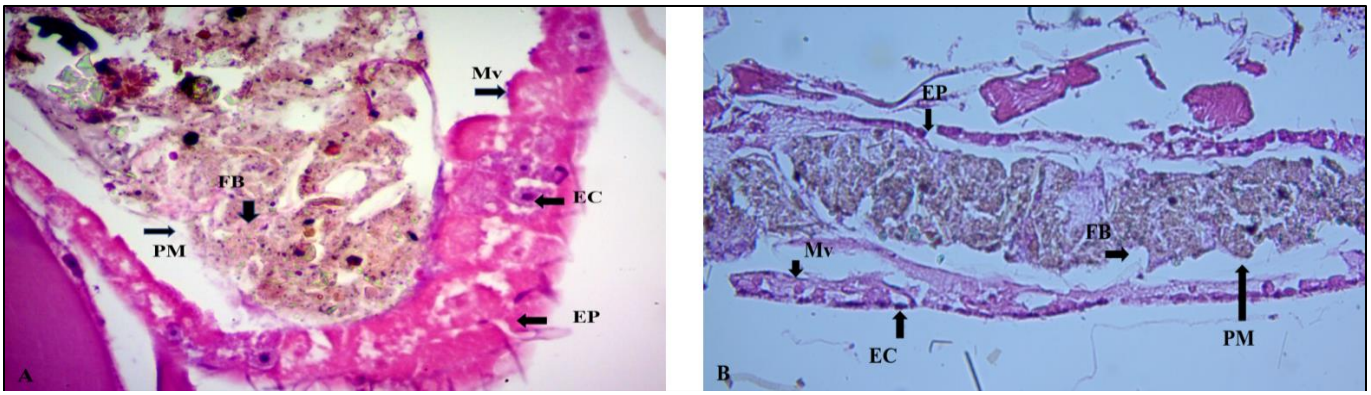


Fig 5: The histopathological midgut of wild-caught *Cx. quinquefasciatus* larvae. The control group (A, 400 x), and the pure camphor group (B, representative of the pure camphor group, 10 x). Food bolus (FB), peritrophic membrane (PM), epithelial layer (EP), epithelial cell (EC), microvilli (Mv).

Table 3: The histopathological results of midgut larvae of wild-caught *Cx. quinquefasciatus* after being exposed to various concentrations of a pure camphor

Treatment	Concentration	N	Histopathological midgut				
			FB	PM	EP	EC	Mv
Control		1	-	-	-	-	-
Pure camphor	0,5	8	+	+	+	+	+
			100% (8/8)	100% (8/8)	100% (8/8)	100% (8/8)	100% (8/8)
	1,5	6	+	+	+	+	+
			100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)
	10,5	5	+	+	+	+	+
			100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)
	25,5	4	+	+	+	+	+
			100% (4/4)	100% (4/4)	100% (4/4)	100% (4/4)	100% (4/4)
	50	4	+	+	+	+	+
			100% (4/4)	100% (4/4)	100% (4/4)	100% (4/4)	100% (4/4)

FB: food bolus, PM: peritrophic membrane; EP: Epithelial layer EC: Epithelial cell Mv: microvilli

4. Discussion

This study demonstrated that pure camphor compounds have larvicidal activity against *Cx. quinquefasciatus* larvae caught in the wild. Pure camphor concentrations of 50 ppm (at 48 h), 10.5 ppm, and 25.5 ppm resulted in 100% mortality of *Cx. quinquefasciatus* larvae (at 72 h). The results of a one-way ANOVA analysis revealed that mortality of *Cx. quinquefasciatus* larvae was significantly different at each pure camphor concentration (p<0.05). Bosley *et al.* [34] supported this study by reporting that rosemary plants containing 56.55% camphor could induce 100% mortality of *Cx. pipiens* larvae at a concentration of 1000 ppm when compared to other plant compounds that did not contain camphor. Furthermore, Ochola *et al.* [35] reported that an oil formulation of *Ocimum kilimandscharicum* emulsified with water (containing 36.6% D-compor) could induce 100% mortality of *Anopheles gambiae* larvae within 48 h with a concentration of 1 ppm.

Camphor is classified as a monoterpenoid (dimers of isoprene that can be divided into acyclic, monocyclic, bicyclic, and tricyclic compounds). The presence of hydroxyl ions (OH-) in monoterpenoids causes oxidative stress, resulting in cell or tissue damage [36]. Terpene compounds, according to Matsuura *et al.* [37], are cytotoxic to animal and human cells, causing damage to plasma membranes, lipid peroxidase, the production of ROS, and mitochondrial damage. Thus, *Cx. quinquefasciatus* larvae mortality was most likely caused by the monoterpenoid compounds found in pure camphor.

At 72 h, the pure camphor had an LC_{50} value of 2.32 ppm and an LC_{90} value of 12.40 ppm, according to this study. When compared to other studies that did not use pure camphor compounds, the level of toxicity of the pure camphor compound showed different LC_{50} and LC_{90} values. Yu *et al.* [38], for example, reported that a *R. officinalis* extract containing camphor and eucalyptol had an LC_{50} value of 38.3 mg/liter for field-strain *Cx. quinquefasciatus* larvae. For *Cx. pipiens* larvae, Bosley *et al.* [34] found that rosemary oil containing camphor had an LC_{50} value of 214.97 ppm and an LC_{90} value of 671.77 ppm. Ochola *et al.* [35] reported LC_{50} values of 0.14 ppm for an emulsified formulation of *Ocimum kilimandscharicum* oil (containing 36.6% D-comphor) showed LC_{50} values of 0.14 ppm and LC_{90} of 0.22 ppm for *An. gambiae* larvae. So, it can be concluded that pure camphor compounds and plants containing camphor compounds have potential as alternative larvicides against mosquito larvae [35].

When *Cx. quinquefasciatus* larvae exposed to pure camphor compounds were compared to controls, AChE activity was inhibited (Fig. 3). Camphor's target is AChE, according to these findings. This study's findings are consistent with those of Ochola *et al.* [35] and Yeom *et al.* [39], who found that camphor is neurotoxic by inhibiting AChE activity at synapses. As a result, large amounts of acetylcholine are deposited at the synapse, paralyzing and killing *Cx. quinquefasciatus* larvae [40]. At a pure camphor concentration of 25.5 ppm, AChE activity was found to be slightly higher than in the control group. The larvae of *Cx. quinquefasciatus* used in this study are a wild strain from the field, which could explain why. According to Brogdon [28], the larvae from the field (wild strain) have two characteristics, namely that the larvae are resistant or susceptible to insecticides. Resistant larvae showed increased AChE activity due to mutation of the knock down resistant gene (KDR) in voltage-gated sodium channel (VGSC), while susceptible larvae showed low AChE activity [41].

Insect GST activity acts as a detoxifying enzyme against exogenous compounds that enter the larvae [42]. When compared to the control group, pure camphor compounds inhibited GST activity in this study. This was due to *Cx. quinquefasciatus* larvae producing more GST to compensate for the camphor treatment after 24, 48, and 72 hours of exposure. The increase in GST activity of *Cx. quinquefasciatus* larvae in this study is consistent with other research studies. For example, Krzyzowski *et al.* [43] found that beetles (*Callosobruchus maculatus*) exposed to camphor on *R. ofcinalis* plants had a significant increase in GST activity. This could be because free radicals at GST concentrations activate GST activity and cause oxidative stress. Furthermore, Quintaneiro *et al.* [44] also reported that camphor causes increased GST activity in zebrafish larvae. Mojarab-Mahboubkar *et al.* [45] reported that camphor in *Artemisia annua* resulted in increased GST activity in fall webworm (*Hyphantria cunea*).

Oxidase is an enzyme with a complex protein in the mitochondrial transmembrane and an oxidative phosphorylation mechanism that functions to produce adenosine triphosphate (ATP). Cytochrome c oxidase is involved in the process of oxygen molecular reduction to water using reducing equivalents donated by cytochrome c and energy binding at site 3 in oxidative phosphorylation.[46] The oxidase activity of *Cx. quinquefasciatus* larvae was lower after exposure to pure camphor compounds than in the control group, according to the study. The discovery that camphor targets oxidase because camphor causes mitochondrial damage, which affects oxidase activity in mitochondria. Satyal *et al.* [7] demonstrated that camphor from the *R.*

officinalis plant acts as a xanthine oxidase inhibitor.

This study demonstrated that pure camphor compounds can cause significant midgut damage in *Cx. quinquefasciatus* larvae. The treatment group showed serious damage to FB, PM, EP, EC, and Mv. This study's findings are consistent with those of other studies. Oftadeh *et al.* [25], for example, reported that *A. annua* flower extract (containing camphor) caused midgut damage in *Glyphodes pyloalis* (a pest of mulberry plants). The elongation and separation of epithelial cells, which causes them to lose their compactness, causes significant damage to the midgut. Camphor compounds cause oxidative stress, which causes midgut damage [35]. Other researchers have reported that any damage to midgut cells can affect the activity of detoxifying enzymes as has been reported by other researchers [25, 26].

There were several limitations to this study, including the following: 1) no material was available to examine esterase activity; 2) no bioassay was performed on female adult *Cx. quinquefasciatus* mosquitoes; 3) did not examine enzyme receptors and biochemical markers such as protein carbonyl and MDA to examine oxidative stress; and 4) there is no correlation between decreased detoxification enzyme activity and histopathological abnormalities in the midgut of *Cx. quinquefasciatus* larvae.

5. Conclusion

Camphor was found to have high larvicidal activity against *Cx. quinquefasciatus* larvae in the field. The LC_{50} of pure camphor was 2.32 ppm and the LC_{90} was 12.40 ppm. Camphor, in its purest form, inhibits or reduces AChE and oxidase activity. Furthermore, pure camphor damages the midgut extensively and severely in all areas (FB, PM, EP, EC, and Mv). According to our findings, pure camphor has the potential to be used as an alternative larvicide to control the population of *Cx. quinquefasciatus* mosquitos.

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8. Competing Interest

The authors declare that they have no competing interest

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