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# Research Article
# Toxic Effects of *Mentha piperita* Extract on *Culex quinquefasciatus* Larvae (Diptera: Culicidae)

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## Abstract

**Background and Objective:** *Mentha piperita* (peppermint) contains terpenoids (essential oils) that can destroy the digestive tracts and nervous systems of insects. The objective of the study was to evaluate the effect of low concentrations of the crude leaf extract of *M. piperita* on *C. quinquefasciatus* larvae through histopathological midgut changes and decreased immunoreactivity of octopamine and tyramine. **Materials and Methods:** The phytochemical analysis of the extract was performed using Gas Chromatography-Mass Spectrometry (GC-MS). *Culex quinquefasciatus* larvae were exposed to different concentrations of the extract. Histopathological changes in the midguts of the larvae were tested by histopathological examination and the immunoreactivity of octopamine and tyramine was measured using an immunohistochemical method. **Results:** Terpenoids were major components of the *M. piperita* crude leaf extract. At 24 hrs, the $LC_{50}$ and $LC_{90}$ values were 2.56 and 6.64 ppm, respectively. The extract caused several histopathological midgut changes, including fragmented food boluses, deformed epithelial cells, disintegrated epithelial layers, damaged microvilli and broken peritrophic membranes. Octopamine and tyramine were detected in the midgut, but their immunoreactivity had decreased. **Conclusion:** *Mentha piperita* has the potential to eradicate the population of *C. quinquefasciatus* as an alternative insecticide.

Key words: *Mentha piperita*, *Culex quinquefasciatus*, octopamine, tyramine, peritrophic membranes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Vector-borne Diseases (VBDs) are serious public health problems across the world, accounting for more than 17% of all infectious diseases and more than 700,000 deaths annually. The causes of VBDs include parasites, bacteria and viruses[1,2]. *Culex quinquefasciatus* is a southern house mosquito that transmits VBDs such as West Nile virus, St. Louis encephalitis, Japanese encephalitis, Rift Valley fever and lymphatic Bancroftian filariasis[3,4]. Thus, mosquito control programs play an important role in inhibiting the transmission of VBDs[5-7].

Recently, *C. quinquefasciatus* mosquitoes have become resistant to insecticides in many countries[8-10]. Some researchers have demonstrated that insecticide-resistant *C. quinquefasciatus* genes exhibit point mutations. For example, an L1014F kdr mutation in the Voltage-gated Sodium Channel (VGSC) gene has been found to confer resistance to pyrethroid and DDT, while a G1195 mutation in the *ace-1* gene has been found to confer resistance to temephos and malathion[9]. One of the factors contributing to insecticide resistance in *C. quinquefasciatus* is the frequent use of insecticides to control mosquito populations[10]. Thus, alternative insecticides obtained from plant bioactive compounds are needed to prevent insecticide resistance in mosquitoes.

*Mentha piperita* L. (peppermint) belongs to the Lamiaceae family and has menthol (monoterpene) as a major bioactive compound[11,12]. Menthol is a type of terpenoid[12]. A previous study demonstrated that *M. piperita* has toxic effects on *C. quinquefasciatus* larvae due to its terpenoid content[13]. Terpenoids comprise a type of essential oil that has toxic effects on insects via contact, ingestion and fumigation by acting on the nervous central system, acetylcholine, γ-aminobutyric acid, the octopaminergic system and the respiratory system[12,14].

Most previous studies of *M. piperita* leaf extract have used high concentrations of extract (~80 ppm) with $LC_{50}$ values ranging from 26.19-111.9 ppm against *Aedes aegypti* larvae for 48-72 hrs[13,15,16]. However, these studies did not investigate the histopathological changes in the midguts of *C. quinquefasciatus* larvae caused by *M. piperita* leaf extract. The present study investigated how low concentrations of this extract (0.05-1 ppm) can kill *C. quinquefasciatus* larvae. For example, the extract can damage the digestive tracts of *C. quinquefasciatus* larvae through histopathological changes in the midgut. Furthermore, the extract can damage components of the nervous system, particularly neurotransmitters, octopamine and tyramine.

A previous study demonstrated that terpenoids (essential oils) can modify the octopaminergic system by competing with octopamine receptors[17,18]. In contrast, present study detected octopamine and tyramine in the midguts of *C. quinquefasciatus* larvae exposed to *M. piperita* leaf extract within 24 hrs. The aim of the present study was to evaluate how low concentrations of the crude leaf extract of *M. piperita* can kill *C. quinquefasciatus* larvae through histopathological changes in the midgut and decreased immunoreactivity of octopamine and tyramine.

## MATERIALS AND METHODS

**Study area:** The study was conducted in April, 2019-2021. Larvae of *C. quinquefasctiatus* were collected in Jakarta city, the capital city of Indonesia. The locations of the larval collection consisted of Jatinegara Sub district, East Jakarta.

***Mentha piperita* crude leaf extraction:** *Mentha piperita* leaves were obtained from a traditional market in East Jakarta, Indonesia. The leaves were cleaned with tap water, cut into small pieces and dried for three weeks at room temperature. Then, the pieces were blended and filtered separately to produce powder samples. Fifty grams of each filtered powder sample were added to an Erlenmeyer tube (500 mL) with 300 mL of absolute methanol and the tubes were kept at room temperature for three days. Next, the methanolic extracts were filtered using filter paper and the sediments were discarded. Finally, the supernatants were evaporated to remove methanol using a vacuum evaporator. The resulting crude extracts were used throughout the entire study.

**Gas chromatography-mass spectrometry (GC-MS) analysis:** To determine the chemical compounds present in the methanolic crude leaf extract of *M. piperita*, a phytochemical screening was conducted using gas chromatography-mass spectrometry (GC-MS; Agilent Technologies, 6890N Network GC System, made in the USA). The procedure and interpretation of the GC-MS analysis were conducted in accordance with the guidelines provided by Agilent Technologies[19]. The GC-MS analysis was conducted at the Forensic Laboratory Centre of Indonesian National Police Headquarters in Jakarta, Indonesia[20].

**Bioassay of *C. quinquefasciatus* larvae:** A larval bioassay procedure was conducted as described previously[21]. The bioassay was conducted using *C. quinquefasciatus* fourth instar larvae collected from several locations in Jakarta,

Indonesia. There were four different concentrations of each extract (0.05, 0.2, 0.5, 0.7 and 1 ppm) modified from a previous study[13]. The larvae were exposed to each of these concentrations separately. In the treatment group, 25 *C. quinquefasciatus* third and fourth instar larvae were added to each plastic cup (200 mL in volume) containing 100 mL of the extract. In the control group, 25 *C. quinquefasciatus* larvae were added to each plastic cup (200 mL in volume) containing tap water. There were four replicates for each treatment group and the bioassay was conducted within 24 hrs to determine the larval mortality rate.

**Histopathological examination of the larval midgut:** The present study used a routine histopathological technique previously described by de Lemos *et al.*[22]. In this procedure, *C. quinquefasciatus* larvae were exposed to the *M. piperita* leaf extract for 24 hrs. All the specimens were fixated with 10% formalin. The dehydration of each specimen was performed using a series of increasing alcohol concentrations (70, 80, 90, 95 and 100%). Then, the specimens were embedded in xylene 1, xylene 2 and xylene 3 solutions and a paraffin block. The resulting blocks were cut (5 µm each) using a manual microtome (Model 320, No. 17664, New York, USA) and feather microtome blades (Feather, S35, Japan). Finally, the sections were stained with hematoxylin and eosin (H and E). The stained specimens were carefully observed under a light microscope and images were taken with a digital microscopic mounted camera (Zeiss Axiocam ERC 5s, Germany).

**Octopamine and tyramine immunohistochemical staining:** An immunohistochemical (IHC) technique was conducted as previously described by Ramos-Vara *et al.*[23]. The IHC staining procedure was performed using diagnostic system kits (Abnova, PAB14697 and Cloud-Clone Corp., PAG048GE01). Briefly, deparaffinization was carried out using xylene 1 and xylene 2 (5 min each) and rehydration was carried out with 100, 96 and 80% alcohol followed by rinsing with distilled water. Next, endogenous peroxidase was quenched with 0.3% $H_2O_2$ in methanol followed by a tap water wash. The sections were then heated with Tris-EDTA buffer (pH 9.0) antigen retrieval using Retrieval Generation 1 (RG1, BIO GEAR, BGRG-0118) for 15 min, chilled at room temperature (15 min) and embedded in PBS solution (3 min). Afterward, nonspecific binding sites were blocked with a background blocker for 5 min. For tyramine, the sections were incubated with the primary antibody Tyramine Polyclonal Antibody (Abnova, PAB14697) 1:1000 overnight at 4°C. For octopamine, the sections were incubated with the primary antibody Polyclonal Antibody to Octopamine (Cloud-Clone Corp., PAG048Ge01) 1:50 overnight at 4°C and then washed with PBS solution. The sections were then incubated with the secondary antibody PolyVue Plus Mouse/Rabbit Enhancer (ten minutes) at room temperature and washed with PBS solution. Next, the sections were incubated with PolyVue Plus HRP Label (10 min) at room temperature and washed with PBS solution. The sections were treated with chromogen substrate and one drop of DAB mixed with 1 mL of DAB buffer, washed with distilled water, treated with hematoxylin, washed with distilled water (3 min) and then treated with one drop of bluing reagent (10 sec). Next, the sections were dehydrated with 80, 96 and 100% alcohol and treated with xylene 1 and xylene 2 for clearing. Finally, the sections were embedded in Entellan (Merck, 1.07961.0500) under glass cover slips.

**Data analysis:** Data were expressed as descriptive statistics and analyzed by statistical package for social sciences (SPSS) ver.20. Data on the mortality rate of the dead larvae were tested for normal distribution (Shapiro-Wilk). Data with normal distribution were analyzed by analysis of variance (ANOVA), while data with non-normal distribution were tested by the Kruskal-Wallis test[24]. The $LC_{50}$ and $LC_{90}$ values were performed by probit analysis with a 95% confidence interval; $p<0.05$ was considered statistically significant[25].

## RESULTS

**Phytochemical analysis of the leaf extract:** The results of the GC-MS analysis showed that the crude leaf extract contained 18 phytochemical compounds. Most of these compounds were terpenoids, followed by fatty acids, alkaloids and vitamin E. The first phytochemical compound detected by GC-MS was the alkaloid 3-(3-methyl-3-oxaziridinyl) pyridine and its area peak was 1.18%. The last phytochemical compound detected by GC-MS was cupreol (a terpenoid). Cupreol was present in the highest concentration (20.35%), while 11,13-dimethyl-12-tetradecen-1-ol acetate (a fatty acid) was present in the smallest concentration (Table 1). The terpenoids in the extract included diterpene (neophytadiene, (Z)-1,3 phytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol), monoterpene (camphene) and steroids (stigmastan-3,5-diene, campesterol and cupreol).

**Larvicidal activity of the leaf extract:** The present study showed that crude leaf extract concentrations ranging from 0.05-1.00 ppm are capable of killing *C. quinquefasciatus* larvae. Table 2 showed the larvicidal activity exhibited by the crude leaf extract. At 24 hrs, the larval mortality of *C. quinquefasciatus* induced by the extract ranged from

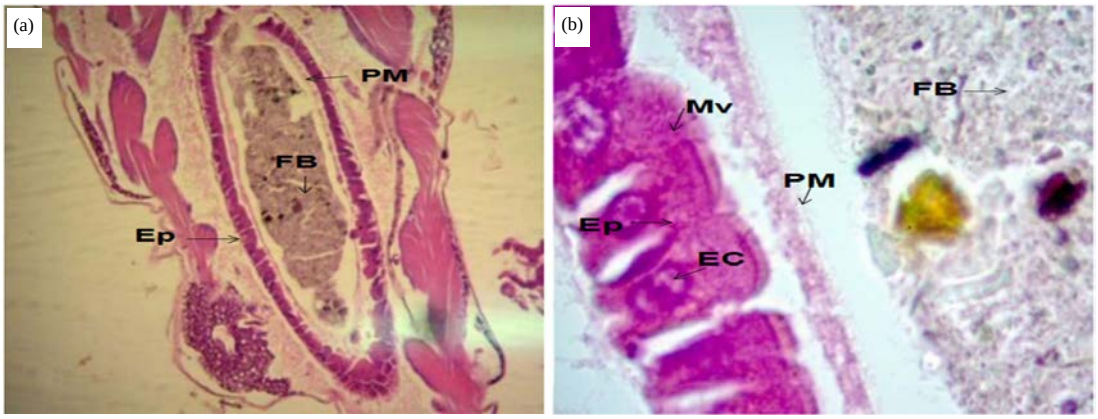


Fig. 1(a-b): Midgut of a healthy *C. quinquefasciatus* larva stained with H and E at (a) 10×magnification and (b) 100×magnification

FB: Food bolus, PM: Peritrophic membrane, Ep: Epithelial layer, EC: Epithelial cell, Mv: Microvilli, H and E: Hematoxylin and eosin

Table 1: GC-MS analysis of the leaf extract

| Number | Real time | Peak | Constituents | Molecular formula | Group |
|---|---|---|---|---|---|
| 1 | 7.05 | 1.18 | 3-(3-Methyl-3-oxaziridinyl)pyridine | $C_7H_8N_2O$ | Alkaloid |
| 2 | 9.05 | 10.47 | Neophytadiene | $C_{20}H_{38}$ | Terpenoid |
| 3 | 9.35 | 2.92 | (Z)-1,3-Phytadiene | $C_{20}H_{38}$ | Terpenoid |
| 4 | 9.98 | 4.38 | Palmitic acid | $C_{16}H_{32}O_2$ | Fatty acid |
| 5 | 10.85 | 6.02 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | $C_{20}H_{40}O$ | Terpenoid |
| 6 | 11.13 | 15.06 | Linolenic acid | $C_{18}H_{30}O_2$ | Fatty acid |
| 7 | 12.68 | 3.49 | Camphene | $C_{10}H_{16}$ | Terpenoid |
| 8 | 12.81 | 2.03 | Dimethylaminoethyl acrylate | $C_7H_{13}NO_2$ | Fatty acid |
| 9 | 12.89 | 1.83 | Methyl 8,11,14-heptadecatrienoate | $C_{18}H_{30}O_2$ | Fatty acid |
| 10 | 13.12 | 4.28 | 2-Palmitoylglycerol | $C_{19}H_{38}O_4$ | Fatty acid |
| 11 | 14.09 | 6.86 | Methyl 8,11,14-heptadecatrienoate | $C_{18}H_{30}O_2$ | Fatty acid |
| 12 | 14.25 | 0.61 | 11,13-Dimethyl-12-tetradecen-1-ol acetate | $C_{19}H_{38}O_4$ | Fatty acid |
| 13 | 14.44 | 6.66 | Oleamide | $C_{18}H_{35}NO$ | Fatty acid |
| 14 | 16.23 | 0.66 | Stigmastan-3,5-diene | $C_{29}H_{48}$ | Terpenoid |
| 15 | 16.35 | 4.70 | Vitamin E | $C_{29}H_{50}O_2$ | Vitamin |
| 16 | 17.22 | 2.33 | Campesterol | $C_{28}H_{48}O$ | Terpenoid |
| 17 | 17.46 | 6.20 | Stigmasterol | $C_{29}H_{48}O$ | Terpenoid |
| 18 | 17.97 | 20.35 | Cupreol | $C_{29}H_{50}O$ | Terpenoid |

Table 2: Larvicidal activity of the leaf extract against *C. quinquefasciatus* larvae

| Concentrations | | *Culex quinquefasciatus* larvae death at 24 hrs | | | $LC_{50}$ | $LC_{90}$ |
|---|---|---|---|---|---|---|
| (ppm) | N | Death | % | Mean±SD | (95% CI) | (95% CI) |
| 0.05 | 125 | 13 | 10.4 | 2.6±0.9 | 2.56 ppm | 6.64 ppm |
| 0.2 | 125 | 18 | 14.4 | 3.6±1.5 | (1.85-15.831) | (3.451-38.001) |
| 0.5 | 125 | 21 | 16.8 | 4.2±0.8 | | |
| 0.7 | 125 | 23 | 18.4 | 4.6±0.5 | | |
| 1.00 | 125 | 26 | 20.8 | 5.2±0.4 | | |

N : Number of *C. quinquefasciatus* larvae tested, LC: Lethal concentration, CI: Confident interval

10.4-20.8%. The $LC_{50}$ and $LC_{90}$ of the extract were 2.56 and 6.64 ppm, respectively. The mean mortality of the *C. quinquefasciatus* larvae increased with increased concentrations of the extract.

**Histopathological damage to the midguts of *C. quinquefasciatus* larvae:** The healthy *C. quinquefasciatus* larvae used as the control group showed that the normal midgut consists of a Food Bolus (FB), a Peritrophic Membrane (PM), an Epithelial Layer (Ep), microvilli and Epithelial Cells (ECs). The Ep is provided by the microvilli and ECs. The ECs are located inside the Ep and may include several degenerative cells (Fig. 1a-b).

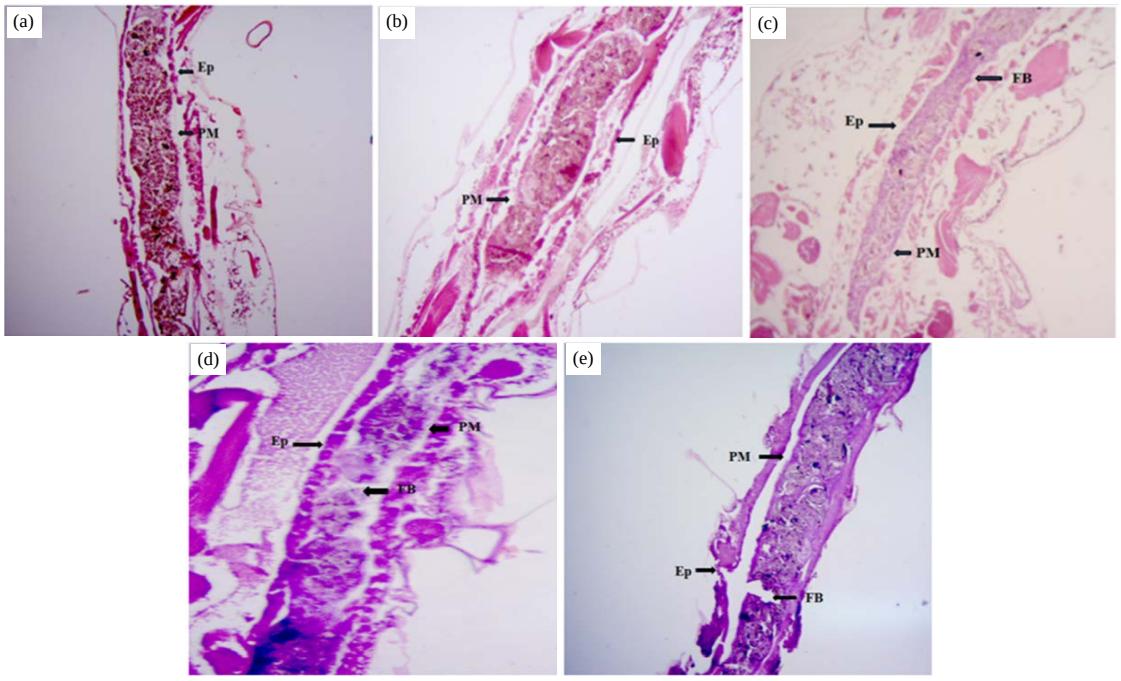


Fig. 2(a-e): Midguts of the *C. quinquefasciatus* larvae were histopathologically damaged by the crude leaf extract at 10×magnification. Extract concentrations (a) 0.05 ppm, (b) 0.2 ppm, (c) 0.5 ppm, (d) 0.7 ppm and (e) 1.00 ppm
Arrow indicated the damaged midgut, FB: Food bolus, PM: Peritrophic membrane, Ep: Epithelial layer, EC: Epithelial cell, Mv: Microvilli

Table 3: Histopathological damage to the midguts of the *C. quinquefasciatus* larvae

| | | Midgut parts of *C. quinquefasciatus* larvae | | | | |
|---|---|---|---|---|---|---|
| Treatment | Concentrations (ppm) | FB | PM | Ep | Mv | EC |
| Control | - | + | + | + | + | + |
| Leaf extract | 0.05 | + | - | - | -/+ | - |
| | 0.2 | + | - | - | - | - |
| | 0.5 | - | - | - | - | - |
| | 0.7 | - | - | - | - | - |
| | 1.00 | - | - | - | - | - |

+ (positive): No histopathological changes in the midgut, - (negative): Histopathological changes in the midgut, FB: Food bolus, PM: Peritrophic membrane, Ep: Epithelial layer, Mv: Microvilli, EC: Epithelial cell

The present study showed that treatment with *M. piperita* crude leaf extract causes damage to the midguts of *C. quinquefasciatus* larvae. At 24 hrs, an extract concentration of 0.05 ppm damaged the Ep of the midgut (Fig. 2a). At an extract concentration of 0.2 ppm, the Ep disintegrated, the ECs ruptured and the microvilli disappeared (Fig. 2b). At extract concentrations of 0.5 ppm (Fig. 2c), 0.7 ppm (Fig. 2d) and 1 ppm (Fig. 2e), all the midgut parts (the FB, PM, Ep, microvilli and ECs) ruptured.

Table 3 summarizes the histopathological damage to the midguts of the *C. quinquefasciatus* larvae caused by the leaf extract. The larvae in the control group were found to have normal midguts, whereas the group treated with the extract showed damage in all parts of the midgut. Increased concentrations of the extract caused increased damage to the midgut.

**Detection of octopamine and tyramine in the midgut:** The present study used the healthy *C. quinquefasciatus* larvae as the control group to evaluate octopamine and tyramine in the midgut of the larvae. Octopamine and tyramine were successfully detected in the midguts of the control larvae. In FB of midgut, octopamine is dark brown in color under the light microscopy, 10× magnifications (Fig. 3a). In Ep of the midgut, octopamine is light brown (small dots) in color (Fig. 3b). In EC of the EP, octopamine is light brown (small dots) under light microscopy, 100 x magnifications (Fig. 3b). In contrast, tyramine is light brown (small dots) only or very light brown in color under the light microscopy, 10×magnifications (Fig. 4a). Sometimes, tyramine is very light brown in color found in FB, Ep and EC using the light microscopy,

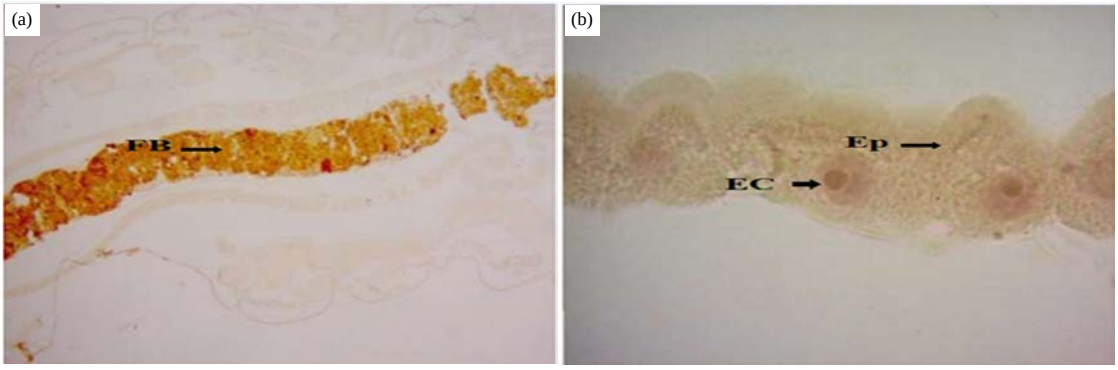


Fig. 3(a-b): Midgut of a *C. quinquefasciatus* larva (control group: where octopamine were detected using an IHC method) at (a) 10×magnification and (b) 100×magnification
Arrow indicates the locations of positive (+) octopamine

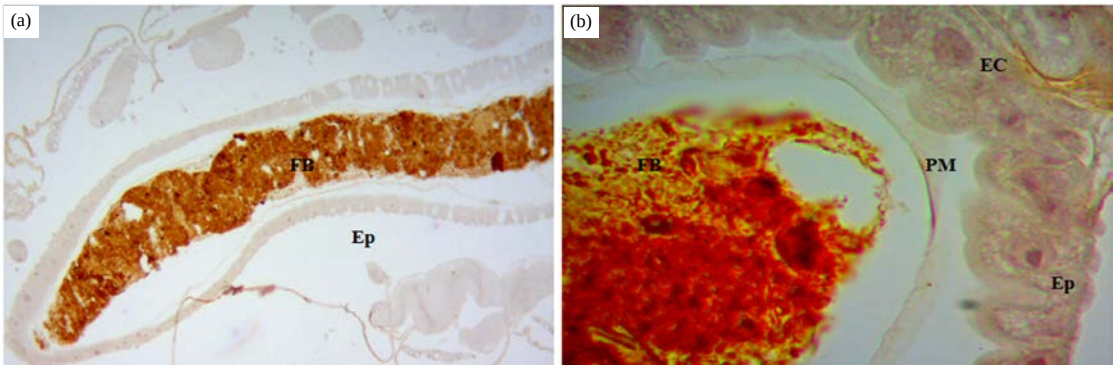


Fig. 4(a-b): Midgut of a *C. quinquefasciatus* larva (control group: where tyramine were detected using an IHC method) at (a) 10×magnification and (b) 100×magnification
Arrow indicates the locations of positive (+) octopamine

Table 4: Immuno reactivity of tyramine in the midguts of *C. quinquefasciatus* larvae

| | | Tyramine in the midgut | | | | |
|---|---|---|---|---|---|---|
| Treatment | Concentrations (ppm) | FB | PM | Ep | Mv | EC |
| Control | - | +++ | - | ++ | - | ++ |
| Leaf extract | 0.05 | + | - | + | - | + |
| | 0.2 | + | - | + | - | - |
| | 0.5 | + | - | + | - | + |
| | 0.7 | + | - | - | - | - |
| | 1 | + | - | - | - | - |

+ (positive): Tyramine was detected in the midgut, - (negative): Tyramine was not detected in the midgut, FB: Food bolus, PM: Peritrophic membrane, Ep: Epithelial layer, Mv: Microvilli, EC: Epithelial cell

100×magnifications (Fig. 4b). The study indicated that octopamine and tyramine were strong immune reactivity in the midgut of the healthy larvae .

Table 4 showed that tyramine was detected by the Polyclonal Antibody to Tyramine using the IHC method. In the control group, tyramine was found in the FB, Ep and ECs. Stronger tyramine immunoreactivity was found in the FB of the midgut than in the Ep and ECs. In contrast, the FB, Ep and ECs showed weak tyramine immunoreactivity after 24 hrs of exposure to the extract. At extract concentrations of 0.7 and 1 ppm, tyramine could not be detected.

In the control group, octopamine was detected in the FB, Ep and ECs. The FB exhibited stronger octopamine immunoreactivity in the midgut than the Ep and ECs. In

Table 5: Immuno reactivity of octopamine in the midguts of *C. quinquefasciatus* larvae

| Treatment | Concentrations (ppm) | Octopamine in the midgut | | | | |
|---|---|---|---|---|---|---|
| | | FB | PM | Ep | Mv | EC |
| Control | - | ++++ | - | +++ | - | +++ |
| Leaf extract | 0.05 | ++ | - | ++ | - | + |
| | 0.2 | ++ | - | + | - | + |
| | 0.5 | ++ | - | + | - | + |
| | 0.7 | ++ | - | - | - | - |
| | 1 | ++ | - | - | - | - |

+ (positive): Octopamine was detected in the midgut, - (negative): Octopamine was not detected in the midgut, FB: Food bolus, PM: Peritrophic membrane, Ep: Epithelial layer, Mv: Microvilli, EC: Epithelial cell

contrast, the FB, Ep and ECs showed weak octopamine immunoreactivity after 24 hrs of exposure to the extract. At extract concentrations of 0.7 and 1 ppm, octopamine could not be detected (Table 5).

## DISCUSSION

The present study employed GC-MS to determine the chemical constituents of *M. piperita* leaf extracts. Most of these extracts contained terpenoids as major compounds, including diterpene (neophytadiene, (Z)-1,3 phytadene and 3,7,11,15-tetramethyl-2-hexadecen-1-ol), monoterpene (camphene) and steroids (stigmastan-3,5-diene, campesterol and cupreol). In contrast, another study found that menthol (monoterpene) is a major component of *M. piperita*[26-28]. In the present study, a vacuum evaporator was used to remove methanol absolute solution, so that there would be no menthol in the extract. Additionally, steroids (particularly cupreol) were found to be major components of the extract. Current findings were consistent with those of Tong[29], who suggested that steroids are a type of terpenoid and that terpenoids, overall are a major component of *M. piperita*[27,28].

In the present study, leaf extract concentrations ranging from 0.05-1.00 ppm exhibited larvicidal activity against *C. quinquefasciatus* larvae. The present study used lower concentrations of extract than those used in previous studies. The larval mortality produced by the extract ranged from 10.4-20.8% and the $LC_{50}$ and $LC_{90}$ of the extract were 2.56 and 6.64 ppm, respectively. These findings, along with the findings of previous studies[13,15], supported the claim that *M. piperita* leaf extract is an efficient larvicidal against mosquito larvae.

Kumar *et al.*[15] reported that an essential oil extracted from *M. piperita* leaves was an efficient larvicide and repellent against the dengue vector *Ae. aegypti*, with $LC_{50}$ and $LC_{90}$ values of 111.9 and 295.18 ppm at 24 hrs of exposure, respectively. Similarly, Kalaivani *et al.*[13] revealed that an oil extract obtained from *M. piperita* was highly toxic against *A. aegypti* larvae, with an $LC_{50}$ value of 47.54 ppm. Kalaivani *et al.*[13] also found that higher rates of larval mortality occurred at higher concentrations (~80 ppm) of extract within 48 hrs of exposure. Another study showed that the $LC_{50}$ value of the extract of *M. piperita* was 26.19 ppm against *A. aegypti* larvae[16]. Similarly, the present study showed that higher rates of larval mortality occurred at higher concentrations (~1 ppm) of extract. Dias and Moraes[11] categorized any plant extract with an $LC_{50}$<100 ppm (mg $L^{-1}$) as an extract containing bioactive compounds. Therefore, the *M. piperita* leaf extract used in the present study can be considered to contain bioactive compounds.

The present study also showed that *M. piperita* leaf extract can damage the histopathological midguts of *C. quinquefasciatus* larvae because the terpenoids in the extract can disrupt cell membranes. This finding was consistent with those of previous studies. Terpenoids are volatile essential oils that penetrate into insects rapidly via the respiratory tract, causing breathing abnormalities that can lead to asphyxiation and, finally, the death of the insect[12,28]. Additionally, terpenoids have been found to induce morphological changes in the midgut[30]. In the present study, the leaf extract caused damage and histopathological changes in midgut parts such as the FB, PM, Ep, microvilli and ECs. According to the results of histopathological examinations using H and E staining, all tested concentrations of the extract were capable of damaging any part of the midgut, but the higher concentrations caused more damage than the lower concentrations. The histopathological changes observed in the midgut were due to the hydrophobic nature of terpenoids. Terpenoids can destroy lipid matrices and can thus destroy the lipid bilayer of the cell membrane, leading to increased fluidity and cell lysis[30]. Therefore, terpenoids contribute to cell membrane disruption and cause damage to the midgut in *C. quinquefasciatus* larvae.

The present study also supported the role of terpenoids in leaf extract-induced oxidative stress[30,31]. Sies[32] reported that oxidative stress, an imbalance of oxidants and antioxidants, causes histopathology. Yu *et al*.[33] demonstrated that increasing hydrogen peroxide, ion hydroxyl, superoxidate and malondialdehyde (MDA) levels leads to histopathology in the midgut structures of *Bombyx mori*. Furthermore, Isah *et al*.[30] reported that sesquiterpenoids cause mitochondrial dysfunction, leading to increased Reactive Oxygen Species (ROS) production via the electron transport chain and thus causing a loss of cellular integrity and parasite death.

The present study demonstrated that the terpenoids in *M. piperita* leaf extract cause low immunoreactivity of the octopamine and tyramine in the midguts of *C. quinquefasciatus* larvae. After 24 hrs of exposure to the leaf extract octopamine and tyramine could be detected in parts of the midgut including the FB, Ep and ECs. In contrast, the immunoreactivity of the octopamine and tyramine in the midguts of the control larvae was strong, while that in the midguts of the larvae exposed to the extract was weak. Therefore, it is possible that the *M. piperita* leaf extract targets neural cells, particularly neurotransmitters (e.g., octopamine and tyramine).

The present findings concerning low immunoreactivity of the octopamine and tyramine in the midguts of *C. quinquefasciatus* larvae were consistent with the theoretical contributions of previous studies. Terpenoids act on the octopaminergic systems of insects[17]. Essential oils from aromatic plants have been found to cause significant increases in both cyclic AMP and calcium levels[17,18]. Terpenoid essential oils can modify the neuronal activity of octopamine receptors because they compete with octopamine by binding to its receptors, which belong to the G-protein receptor family[18,34]. Additionally, terpenoid essential oils have a nervous cell target octopamine (a neurotransmitter) through which they can cause physiological modulations in insects[12]. Octopamine and tyramine are known as physiological behavior markers in insects, as these neurotransmitters play important roles in feeding, flying, growth and development[34,35]. Therefore, it appears that the *M. piperita* leaf extract leads to physiological modulations and, finally, death in *C. quinquefasciatus* larvae by targeting the nervous system.

The present study supported the usefulness of *M. piperita* leaf extract in reducing environmental pollution and preventing the development of insecticide resistance in *C. quinquefasciatus* and other mosquitoes. *Culex quinquefasciatus*, known as vector of VBDs is dangerous to human life. Many people live in the endemic areas of VBDs[1,2] and *C. quinquefasciatus* has become resistant to insecticides in many countries[10]. Therefore, the findings of the present study will aid in reducing the application of synthetic insecticides. Essential oils act at multiple levels in insects, so the probability that insects will generate resistance to these compounds is low[36,37]. Iwuagwu *et al*.[38] suggested that the use of plant-derived bioactive compounds is an eco-friendly way to control various vectors of disease.

## CONCLUSION

*Mentha piperita* leaf extract was found to contain terpenoids, fatty acids, alkaloids and vitamin E. Low concentrations of the extract exhibited larvicidal activity against *C. quinquefasciatus* larvae. Terpenoids, which are major components of this extract, have targets of toxicity in the digestive tract (the midgut) and the nervous system (octopamine and tyramine). Therefore, *M. piperita* leaf extract could serve as an alternative insecticide to control the *C. quinquefasciatus* population.

## SIGNIFICANCE STATEMENT

Low concentrations of *M. piperita* leaf extract showed larvicidal activity against *C. quinquefasciatus* larvae. Terpenoids, which are major components of this extract, have targets of toxicity in the digestive tract (the midgut) and the nervous system (octopamine and tyramine). Thus, *M. piperita* leaf extract could serve as an alternative insecticide to control the *C. quinquefasciatus* population.

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