# CHAPTER 3

# Results

## 3.1 Preparation of plant products

Extraction of seventeen plant materials provided essential oils and/or ethanolic extracts with varying yields and different physical characteristics (Table 3.1). In essential oil isolation, only nine plants, including *Myristica fragrans*, *Piper sarmentosum*, *Limnophila aromatica*, *Coriandrum sativum*, *Foeniculum vulgare*, *Petroselinum crispum*, *Amomum uliginosum*, *Curcuma longa*, and *Kaempferia pandurata* yielded from 0.32 to 2.01% oils according to dry weight (v/w). These volatile oils were less dense than water, clear, and yellow or brown, with a characteristic smell. Ethanolic extractions provided plant extracts with varied yields that ranged in dry weight from 4.39 to 54.25% (w/w). The highest yields were obtained from *M. fragrans* in both essential oils (2.01%, v/w) and ethanolic extracts (54.25%, w/w), whereas *P. sarmentosum* (0.32%, v/w) and *C. sativum* (4.39%, w/w) provided the lowest yields of essential oil and ethanolic extract, respectively.

**Table 3.1** Percentage yield (% Yield) and physical characteristics (color, phase, and density) of plant products, including essential oils and ethanolic extracts.

| Plant | Plant product | | | | | | |
|---|---|---|---|---|---|---|---|
| | Essential oil | | | | Ethanolic extract | | |
| | % Yield | Color | Phase | Density (g/ml) | % Yield | Color | Phase |
| *B. lacera* | 0.0 | ND | ND | ND | 11.12 | Dark green | Semi-solid |
| *R. sativus* | 0.0 | ND | ND | ND | 18.14 | Dark yellow | Semi-solid |
| *P. odoratum* | 0.0 | ND | ND | ND | 13.30 | Dark green | Powder |
| *M. fragrans* | 2.01 | Light yellow | Liquid | 0.98 | 54.25 | Orange | Semi-solid |
| *P. sarmentosum* | 0.32 | Brown | Liquid | 0.99 | 11.60 | Dark green | Semi-solid |
| *L. aromatica* | 1.53 | Light yellow | Liquid | 0.92 | 10.47 | Dark green | Semi-solid |
| *P. kurroa* | 0.0 | ND | ND | ND | 43.42 | Yellow | Powder |
| *S. aculeatissimum* | 0.0 | ND | ND | ND | 12.09 | Dark green | Semi-solid |
| *S. indicum* | 0.0 | ND | ND | ND | 25.23 | Green | Powder |
| *C. sativum* | 1.17 | Pale yellow | Liquid | 0.85 | 4.39 | Brown | Liquid |
| *F. vulgare* | 0.67 | Pale yellow | Liquid | 0.97 | 7.94 | Brown | Liquid |
| *P. crispum* | 1.74 | Pale yellow | Liquid | 0.89 | 12.26 | Dark green | Semi-solid |
| *A. uliginosum* | 0.93 | Light yellow | Liquid | 0.91 | 5.46 | Brown | Semi-solid |
| *C. aeruginosa* | 0.0 | ND | ND | ND | 9.55 | Dark yellow | Semi-solid |
| *C. longa* | 0.77 | Pale yellow | Liquid | 0.93 | 13.04 | Yellow | Semi-solid |
| *K. pandurata* | 0.57 | Light yellow | Liquid | 0.97 | 14.48 | Dark yellow | Semi-solid |
| *K. parviflora* | 0.0 | ND | ND | ND | 8.57 | Black | Powder |

ND: Not determined, as no essential oil was obtained from this plant species

## 3.2 Investigation of insecticidal activity of plant products

### 3.2.1 Preliminary screening for larvicidal activity against *Ae. aegypti*

Preliminary laboratory trials for screening larvicidal activity of the plant products against the MCM-S *Ae. aegypti* strain revealed a wide variety of toxicity. At a 100 ppm concentration, only the essential oils of *P. crispum*, *F. vulgare*, *M. fragrans*, *L. aromatica*, *P. sarmentosum*, and *C. longa*, as well as *M. fragrans* ethanolic extract, produced promising efficacy in the range of 90 to 100% larval mortality (Table 3.2). The remaining plant products offered no or little larval mortality ranging from 0 to 36%. The control and untreated groups showed no conspicuous effect, with zero larval mortality.

### 3.2.2 Dose-response larvicidal bioassay

The essential oils of *P. crispum*, *F. vulgare*, *M. fragrans*, *L. aromatica*, *P. sarmentosum*, *C. longa*, and *M. fragrans* ethanolic extract that considered as the effective agents were studied further in a dose-response larvicidal bioassay against MCM-S *Ae. aegypti*. The lethal concentrations ($LC_{50}$, $LC_{95}$, and $LC_{99}$), together with their 95% confidence intervals of dose-mortality bioassays, are demonstrated in Table 3.3. The results revealed that the mortality of larvae was dose dependent. There was an increase in larval mortality (16.50 to 90.0%) when dosages of the test samples were increased from 35.60 to 100 ppm. The highest efficacy established from *P. crispum* oil ($LC_{50}$ value = 43.22 ppm), followed by the oils of *F. vulgare* ($LC_{50}$ value = 44.84 ppm), *M. fragrans* ($LC_{50}$ value = 47.42 ppm), *L. aromatica* ($LC_{50}$ value = 47.94 ppm), *P. sarmentosum* ($LC_{50}$ value = 49.19 ppm), *C. longa* ($LC_{50}$ value = 65.51 ppm), and *M. fragrans* ethanolic extract ($LC_{50}$ value = 75.45 ppm). Therefore, *P. crispum* oil that provided the highest larvicidal activity was selected for further studies, including chemical analysis, antimosquito assessment, and determination of mosquitocidal actions against both larval and adult stages of MCM-S, PMD-R, and UPK-R strains of *Ae. aegypti*.

**Table 3.2** Larvicidal activity of plant products, including essential oils and ethanolic extracts, against the pyrethroid susceptible (MCM-S) strain of *Ae. aegypti*

| **Plant** | **Thai name** | **Plant product** | |
|---|---|---|---|
| | | **Essential oil** | **Ethanolic extract** |
| | | **% Mortality** | **% Mortality** |
| *B. lacera* | ผักกาดนา | ND | 0 |
| *R. sativus* | ผักขี้หูด | ND | 0 |
| *P. odoratum* | ผักไผ่ | ND | 0 |
| *M. fragrans* | จันทน์เทศ | 100 | 90 |
| *P. sarmentosum* | ชะพลู | 100 | 36 |
| *L. aromatica* | ผักแขยง | 100 | 0 |
| *P. kurroa* | โกฐก้านพร้าว | ND | 0 |
| *S. aculeatissimum* | มะเขือขื่น | ND | 0 |
| *S. indicum* | มะแว้งต้น | ND | 0 |
| *C. sativum* | ผักชี | 0 | 0 |
| *F. vulgare* | เทียนแกลบ | 100 | 0 |
| *P. crispum* | เทียนเยาวพาณี | 100 | 0 |
| *A. uliginosum* | เร่ว | 16 | 0 |
| *C. aeruginosa* | มหาเมฆ | ND | 0 |
| *C. longa* | ขมิ้นชัน | 100 | 0 |
| *K. pandurata* | กระชาย | 0 | 20 |
| *K. parviflora* | กระชายดำ | ND | 0 |

**Table 3.3** Larvicidal activity of plant products derived from six selected plant species against the pyrethroid susceptible (MCM-S) strain of *Ae*. *aegypti*

| Concentration of plant product (ppm) | % Mortality (Mean±SE) | Larvicidal activity (95% CI, ppm) | | | Slope values±SE |
|---|---|---|---|---|---|
| | | $LC_{50}$ | $LC_{95}$ | $LC_{99}$ | |
| *M. fragrans* (EE) | | 75.45 | 123.60 | 151.65 | 7.67±0.40 |
| 60.00 | 24.50±6.24 | (74.07-76.80) | (118.09-130.63) | (142.30-163.89) | |
| 70.00 | 38.25±11.62 | | | | |
| 80.00 | 56.00±12.46 | | | | |
| 90.00 | 70.50±4.51 | | | | |
| 100.00 | 85.25±2.87 | | | | |
| *M. fragrans* (EO) | | 47.42 | 69.28 | 81.07 | 9.99±0.51 |
| 38.40 | 18.25±2.75 | (46.77-48.07) | (66.81-72.39) | (77.11-86.14) | |
| 43.20 | 35.25±5.56 | | | | |
| 48.00 | 51.25±2.99 | | | | |
| 52.80 | 65.25±2.63 | | | | |
| 57.60 | 82.25±3.40 | | | | |
| *P. sarmentosum* (EO) | | 49.19 | 75.10 | 89.49 | 8.95±0.40 |
| 39.60 | 22.75±3.30 | (48.47-49.88) | (72.56-78.21) | (85.30-94.73) | |
| 44.55 | 33.25±2.06 | | | | |
| 49.50 | 50.00±0.82 | | | | |
| 54.45 | 63.00±0.82 | | | | |
| 59.40 | 74.75±2.22 | | | | |
| 64.35 | 89.00±4.55 | | | | |
| *L. aromatica* (EO) | | 47.94 | 65.14 | 73.96 | 12.35±0.58 |
| 44.10 | 24.25±0.96 | (47.37-48.49) | (63.51-67.13) | (71.39-77.15) | |
| 46.00 | 38.25±4.99 | | | | |
| 50.60 | 60.00±2.83 | | | | |
| 52.20 | 77.00±3.37 | | | | |
| 59.80 | 90.00±3.56 | | | | |
| *F. vulgare* (EO) | | 44.84 | 57.05 | 63.03 | 15.74±0.90 |
| 41.70 | 30.75±6.99 | (44.45-45.21) | (55.78-58.65) | (61.04-65.60) | |
| 43.70 | 45.25±11.15 | | | | |
| 45.60 | 52.50±6.24 | | | | |
| 47.50 | 65.00±12.68 | | | | |
| 49.50 | 73.75±10.87 | | | | |
| 51.40 | 84.00±4.08 | | | | |
| *P. crispum* (EO) | | 43.22 | 66.60 | 79.66 | 8.76±0.40 |
| 35.60 | 22.50±1.29 | (42.56-43.86) | (64.34-69.37) | (75.89-84.40) | |
| 40.05 | 39.75±1.26 | | | | |
| 44.50 | 54.75±1.71 | | | | |
| 48.95 | 67.50±1.29 | | | | |
| 53.40 | 77.25±0.96 | | | | |
| 57.85 | 88.00±0.82 | | | | |
| *C. longa* (EO) | | 65.51 | 110.93 | 137.98 | 7.19±0.36 |
| 46.50 | 16.50±1.29 | (64.28-66.78) | (105.39-117.99) | (128.69-150.12) | |
| 55.80 | 28.75±3.40 | | | | |
| 65.10 | 47.50±1.91 | | | | |
| 74.40 | 63.25±1.26 | | | | |
| 83.70 | 81.00±2.94 | | | | |

### 3.2.3 Antimosquito bioassays of *P. crispum* oil and synthetic insecticidal compounds

#### 3.2.3.1 Larvicidal bioassay

The larvicidal activity of *P. crispum* oil against MCM-S, PMD-R, and UPK-R was found to be comparatively effective, with an insignificant difference of $LC_{50}$ values of 43.22, 44.50, and 44.03 ppm, respectively (Table 3.4 and Figure 3.1). Conversely, the extremely different degrees in response to commercial synthetic insecticides between pyrethroid susceptible and resistant *Ae. aegypti* strains were documented in both larval and adult stages. Larval susceptibility among the three strains of *Ae. aegypti*; i.e., MCM-S, PMD-R, and UPK-R, was markedly different, with $LC_{50}$ values of 3.54, 6.62, and 6.89 ppb, respectively, for temephos; 1.46, 5.97, and 417.59 ppb, respectively, for permethrin; and 0.16, 1.50, and 9.37 ppb, respectively, for deltamethrin (Table 3.5 and Figure 3.2).

#### 3.2.3.2 Adulticidal bioassay

A similar level of susceptibility to *P. crispum* oil was also observed in MCM-S, PMD-R, and UPK-R adults, with insignificantly different $LD_{50}$ values of 6.01, 6.15, and 6.12 µg/mg female, respectively (Table 3.6 and Figure 3.3). However, distinct differences in response to commercial synthetic insecticides between pyrethroid susceptible and resistant *Ae. aegypti* strains were also documented in the adult stage. Adulticidal activity of insecticides against MCM-S, PMD-R, and UPK-R were significantly different, with $LD_{50}$ values of 0.47, 3.81, and 29.36 ng/mg female, respectively, for permethrin; and 0.06, 3.66, and 7.10 ng/mg female, respectively, for deltamethrin (Table 3.7 and Figure 3.4).

**Table 3.4** Larvicidal activity of *P. crispum* oil against the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti*

| Mosquito/Oil dosage | % Mortality (Mean±SE) | Larvicidal activity (95% CI, ppm) | | | Slope values±SE |
|---|---|---|---|---|---|
| | | $LC_{50}$ | $LC_{95}$ | $LC_{99}$ | |
| MCM-S | | 43.22 | 66.60 | 79.66 | 8.76±0.40 |
| 35.60 | 22.50±1.29 | (42.56-43.86) | (64.34-69.37) | (75.89-84.40) | |
| 40.05 | 39.75±1.26 | | | | |
| 44.50 | 54.75±1.71 | | | | |
| 48.95 | 67.50±1.29 | | | | |
| 53.40 | 77.25±0.96 | | | | |
| 57.85 | 88.00±0.82 | | | | |
| PMD-R | | 44.50 | 68.29 | 81.55 | 8.84±0.40 |
| 35.60 | 19.00±0.82 | (43.85-45.13) | (65.91-71.22) | (77.63-86.47) | |
| 40.05 | 35.00±0.82 | | | | |
| 44.50 | 51.25±1.71 | | | | |
| 48.95 | 63.25±0.96 | | | | |
| 53.40 | 74.25±1.71 | | | | |
| 57.85 | 85.50±1.00 | | | | |
| UPK-R | | 44.03 | 67.71 | 80.92 | 8.80±0.40 |
| 35.60 | 17.25±0.96 | (43.38-44.67) | (65.37-70.59) | (77.05-85.79) | |
| 40.05 | 38.25±2.63 | | | | |
| 44.50 | 54.50±2.38 | | | | |
| 48.95 | 67.25±2.22 | | | | |
| 53.40 | 76.50±1.29 | | | | |
| 57.85 | 83.00±1.83 | | | | |

**Table 3.5** Larvicidal activity of temephos, permethrin, and deltamethrin against the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti*

| Insecticide/ Mosquito | % Mortality (Mean±SE) | Larvicidal activity (95% CI, ppb) | | | Slope values±SE |
|---|---|---|---|---|---|
| | | $LC_{50}$ | $LC_{95}$ | $LC_{99}$ | |
| Temephos | | | | | |
| MCM-S | | 3.54 | 5.73 | 6.99 | 7.86±0.52 |
| 3.00 | 29.25±1.50 | (3.47-3.61) | (5.43-6.14) | (6.49-7.71) | |
| 3.50 | 47.50±1.29 | | | | |
| 4.00 | 65.75±2.36 | | | | |
| 4.50 | 80.00±1.41 | | | | |
| PMD-R | | 6.62 | 10.57 | 12.84 | 8.08±0.31 |
| 5.00 | 20.75±2.75 | (6.19-7.00) | (10.11-11.77) | (12.27-14.74) | |
| 6.00 | 34.25±2.06 | | | | |
| 7.00 | 54.25±2.22 | | | | |
| 8.00 | 70.50±1.91 | | | | |
| 9.00 | 85.50±2.65 | | | | |
| UPK-R | | 6.89 | 10.83 | 13.07 | 8.37±0.32 |
| 6.00 | 27.25±0.96 | (6.54-7.23) | (10.36-11.85) | (12.43-14.66) | |
| 7.00 | 48.25±0.50 | | | | |
| 8.00 | 68.00±1.41 | | | | |
| 9.00 | 84.50±1.00 | | | | |
| Permethrin | | | | | |
| MCM-S | | 1.46 | 1.97 | 2.23 | 12.58±0.52 |
| 1.30 | 27.75±1.50 | (1.44-1.47) | (1.93-2.01) | (2.17-2.30) | |
| 1.40 | 42.75±2.22 | | | | |
| 1.50 | 56.00±2.83 | | | | |
| 1.60 | 66.75±0.96 | | | | |
| 1.70 | 79.00±0.82 | | | | |
| 1.80 | 89.50±1.29 | | | | |
| PMD-R | | 5.97 | 18.24 | 28.98 | 3.39±0.18 |
| 4.00 | 30.50±1.91 | (5.69-6.23) | (16.55-20.54) | (25.20-34.40) | |
| 6.00 | 47.50±2.65 | | | | |
| 8.00 | 64.25±1.50 | | | | |
| 10.00 | 77.25±0.96 | | | | |
| 12.00 | 87.50±1.00 | | | | |
| UPK-R | | 417.59 | 998.49 | 1432.77 | 4.34±0.20 |
| 300 | 29.25±2.99 | (403.14-431.25) | (936.16-1077.45) | (1306.09-1599.83) | |
| 400 | 46.50±1.73 | | | | |
| 500 | 59.50±3.11 | | | | |
| 600 | 72.25±3.50 | | | | |
| 700 | 85.00±4.24 | | | | |
| 800 | 91.50±1.29 | | | | |
| Deltamethrin | | | | | |
| MCM-S | | 0.16 | 0.34 | 0.42 | 3.87±0.23 |
| 0.10 | 23.50±1.73 | (0.15-0.16) | (0.31-0.38) | (0.38-0.48) | |
| 0.15 | 45.00±2.58 | | | | |
| 0.20 | 61.75±2.99 | | | | |
| 0.25 | 81.00±1.41 | | | | |
| PMD-R | | 1.50 | 4.85 | 7.87 | 3.24±0.23 |
| 1.00 | 29.50±2.08 | (1.43-1.58) | (4.23-5.79) | (6.50-10.14) | |
| 1.50 | 49.00±1.83 | | | | |
| 2.00 | 61.25±3.30 | | | | |
| 2.50 | 79.50±1.73 | | | | |
| UPK-R | | 9.37 | 18.84 | 25.17 | 5.42±0.22 |
| 6.00 | 15.25±0.96 | (9.13-9.60) | (17.93-19.95) | (23.49-27.26) | |
| 8.00 | 35.50±1.00 | | | | |
| 10.00 | 57.00±3.46 | | | | |
| 12.00 | 69.00±2.16 | | | | |
| 14.00 | 82.00±0.82 | | | | |
| 16.00 | 91.75±1.26 | | | | |

**Table 3.6** Adulticidal activity of *P. crispum* oil against the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti*

| Mosquito/Oil dosage | % Mortality (Mean±SE) | Adulticidal activity (95% CI, µg/mg female) | | | Slope values±SE |
|---|---|---|---|---|---|
| | | $LD_{50}$ | $LD_{95}$ | $LD_{99}$ | |
| MCM-S | | 6.01 | 9.39 | 11.30 | 8.48±0.37 |
| 4.45 | 15.25±2.99 | (5.91-6.11) | (9.05-9.82) | (10.74-12.01) | |
| 5.34 | 32.75±4.19 | | | | |
| 6.23 | 51.25±2.99 | | | | |
| 7.12 | 72.50±4.73 | | | | |
| 8.01 | 88.25±6.75 | | | | |
| PMD-R | | 6.15 | 9.82 | 11.92 | 8.09±0.37 |
| 4.45 | 14.00±2.94 | (6.04-6.25) | (9.42-10.31) | (11.26-12.75) | |
| 5.34 | 30.50±4.80 | | | | |
| 6.23 | 50.25±1.50 | | | | |
| 7.12 | 68.25±4.79 | | | | |
| 8.01 | 84.50±4.65 | | | | |
| UPK-R | | 6.12 | 9.93 | 12.14 | 7.83±0.36 |
| 4.45 | 14.75±2.63 | (6.01-6.23) | (9.51-10.46) | (11.44-13.03) | |
| 5.34 | 32.75±2.63 | | | | |
| 6.23 | 49.75±2.36 | | | | |
| 7.12 | 69.00±2.58 | | | | |
| 8.01 | 83.75±3.30 | | | | |

**Table 3.7** Adulticidal activity of permethrin and deltamethrin against the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti*

| Insecticide/ Mosquito | % Mortality (Mean±SE) | Adulticidal activity (95% CI, ng/mg female) | | | Slope values±SE |
|---|---|---|---|---|---|
| | | $LD_{50}$ | $LD_{95}$ | $LD_{99}$ | |
| Permethrin | | | | | |
| MCM-S | | 0.47 | 1.04 | 1.45 | 4.75±0.31 |
| 0.30 | 19.25±0.96 | (0.46-0.49) | (0.94-1.19) | (1.27-1.73) | |
| 0.40 | 35.00±1.83 | | | | |
| 0.50 | 52.75±2.06 | | | | |
| 0.60 | 71.75±2.22 | | | | |
| PMD-R | | 3.81 | 14.47 | 25.14 | 2.84±0.16 |
| 2.00 | 23.00±1.83 | (3.60-4.03) | (12.74-16.90) | (21.05-31.29) | |
| 4.00 | 49.75±1.50 | | | | |
| 6.00 | 68.75±1.26 | | | | |
| 8.00 | 85.00±0.82 | | | | |
| UPK-R | | 29.36 | 47.78 | 58.46 | 7.78±0.46 |
| 25.00 | 31.50±1.30 | (28.71-29.96) | (45.57-50.67) | (54.67-63.59) | |
| 30.00 | 50.50±1.73 | | | | |
| 35.00 | 69.75±1.50 | | | | |
| 40.00 | 87.75±2.63 | | | | |
| Deltamethrin | | | | | |
| MCM-S | | 0.06 | 0.22 | 0.37 | 3.06±0.16 |
| 0.03 | 17.50±0.58 | (0.06-0.07) | (0.20-0.26) | (0.31-0.46) | |
| 0.05 | 35.50±1.73 | | | | |
| 0.07 | 50.75±2.22 | | | | |
| 0.09 | 65.75±1.50 | | | | |
| 0.11 | 80.00±1.41 | | | | |
| PMD-R | | 3.66 | 13.93 | 24.24 | 2.83±0.16 |
| 2.00 | 24.50±1.29 | (3.45-3.87) | (12.29-16.22) | (20.34-30.09) | |
| 4.00 | 52.00±3.16 | | | | |
| 6.00 | 70.25±1.50 | | | | |
| 8.00 | 86.25±3.30 | | | | |
| UPK-R | | 7.10 | 31.15 | 57.49 | 2.56±0.18 |
| 4.00 | 28.00±2.16 | (6.73-7.47) | (26.11-39.20) | (44.88-79.46) | |
| 6.00 | 42.25±1.50 | | | | |
| 8.00 | 51.75±1.71 | | | | |
| 10.00 | 62.50±1.29 | | | | |
| 12.00 | 76.25±0.96 | | | | |

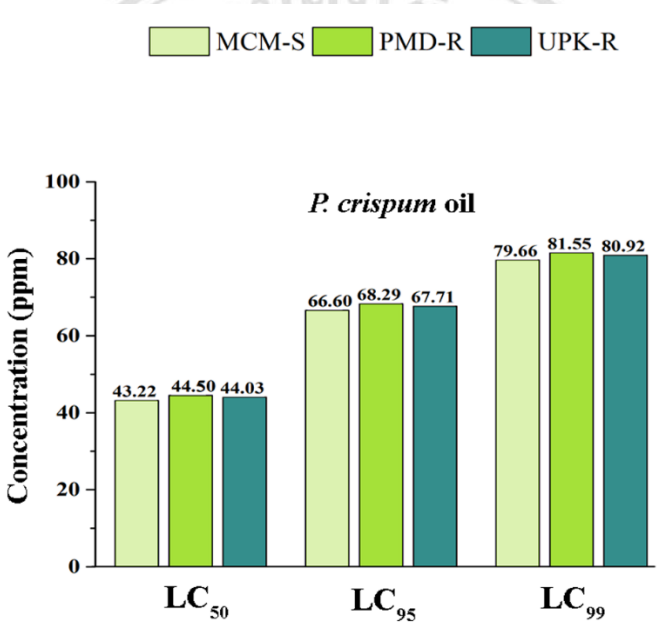


**Figure 3.1** Larvicidal activity of *P. crispum* oil against the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti*.

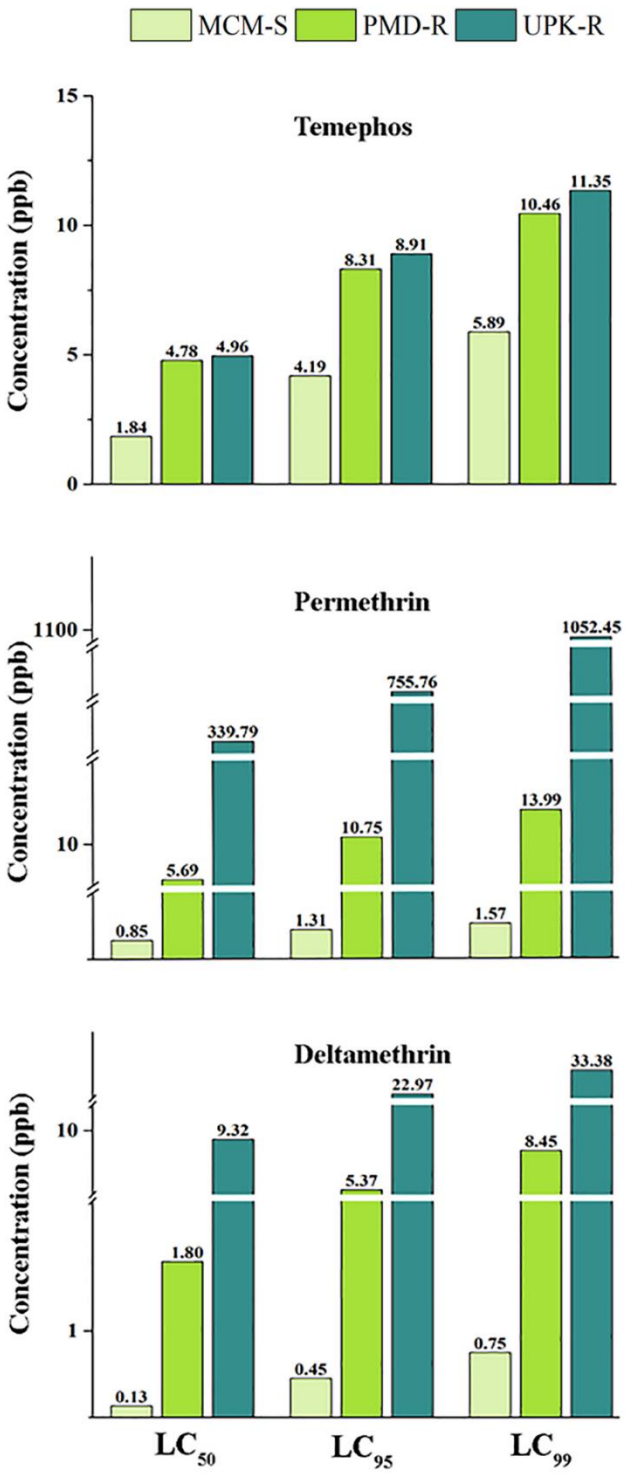


**Figure 3.2** Larvicidal activity of temephos, permethrin, and deltamethrin against the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti*.

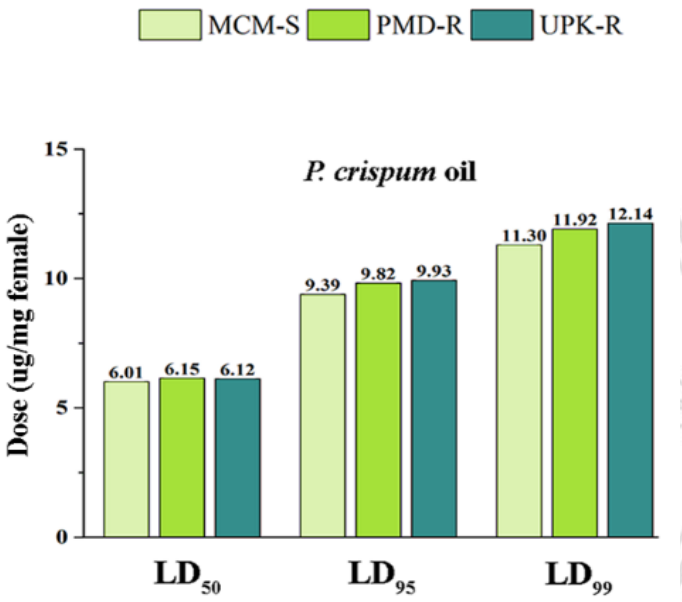


**Figure 3.3** Adulticidal activity of *P. crispum* oil against the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti*.

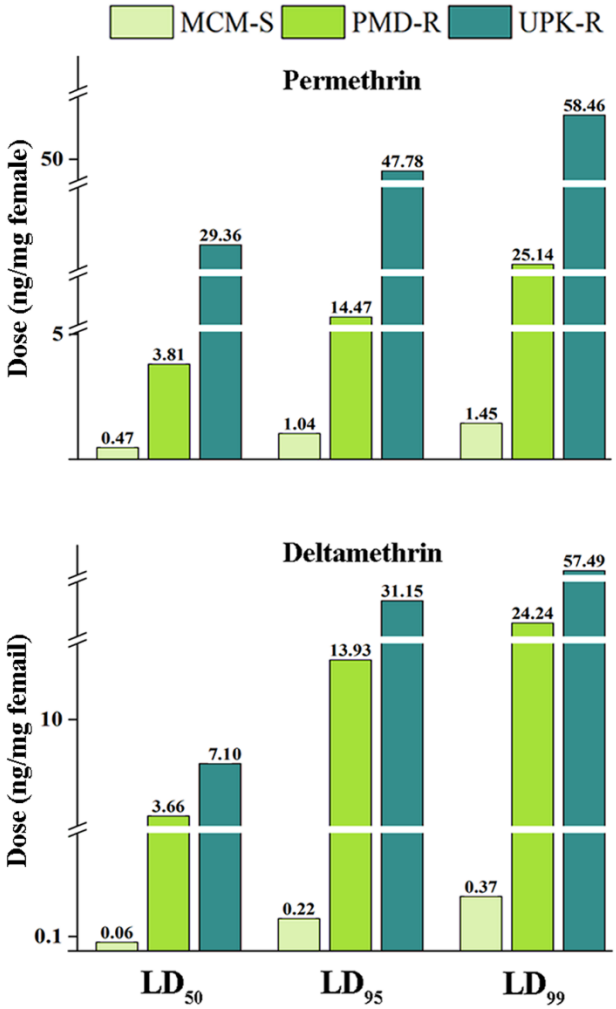




**Figure 3.4** Adulticidal activity of permethrin, and deltamethrin against the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti*.

### 3.3 Determination of mosquitocidal actions of *P. crispum* oil

#### 3.3.1 Behavioral response observation

##### 3.3.1.1 Larvae

Behavioral observations on mosquitoes treated with discriminating concentration ($LC_{99}$) of *P. crispum* oil showed similar pattern of abnormal behaviors in the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti* larvae. The abnormal behaviors were divided into 6 phases as demonstrated in Table 3.8. After immediate exposure, all strains of the mosquito larvae were active and exhibited in a normal appearance, siphon rested up, and head hung down. Initial abnormal excitation and restlessness were observed after 1-2 min of exposure. Between 2-10 min after exposure some of larvae were restless, frequently sank down and quickly floated up with coiling movement. Subsequently, the restlessness was still persisted and more toxic symptoms such as tremors and convulsions at the bottom of the container were observed with approximately 2-5 larvae. Similar evidence of restlessness, tremors, convulsions followed by paralysis was observed at 20-30 min of exposure. More than 50% of the larvae were paralyzed and sunk to the bottom of the bowl after 50-60 min of treatment. Thereafter, all of them died within 6 h.

##### 3.3.1.2 Adults

The similarly behavioral changes were observed on the MCM-S, PMD-R, and UPK-R adult females of *Ae. aegypti* after treated with *P. crispum* oil at the dose of $LD_{99}$ value. The results found that the treated females were restless followed by paralyzed and died immediately after 3 min of exposure.

**Table 3.8** Behavioral changes of the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti* larvae after treatment with $LC_{99}$ of *P. crispum* oil

| Phase | Signs and symptoms | Time of initiation of symptoms after exposure | | |
|---|---|---|---|---|
| | | MCM-S (79.66 ppm) | PMD-R (81.55 ppm) | UPK-R (80.92 ppm) |
| 1 | Initial abnormal excitation and restlessness | 1-2 min | 1-2 min | 1-2 min |
| 2 | Restless, frequently sank down and quickly floated up with coiling movement | 2-10 min | 2-10 min | 2-10 min |
| 3 | Restlessness, tremor, and convulsion at the bottom of bowl (2-5 larvae) | 10-15 min | 15-20 min | 15-20 min |
| 4 | Restlessness, tremor, and convulsion followed by paralysis | 20 min | 30 min | 30 min |
| 5 | 50% of the larvae paralyzed and sunk to the bottom of the bowl | 50 min | 60 min | 60 min |
| 6 | 100% mortality | 6 h | 6 h | 6 h |

### 3.3.2 Physical change observation

#### 3.3.2.1 Light microscopic (LM) study

##### 3.3.2.1.1 Larvae

Morphological alterations of the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti* larvae after exposure to *P. crispum* oil (treated group) and dimethyl sulfoxide-distilled water (control group) were observed under LM. In general, the control and treated larvae exhibited similarities in morphological features and cuticular sculpturing of the body segments (head, thorax, and abdomen) and other organs such as eyes, antennae, mouth brushes, setae, saddle, and siphon. A distinct difference, however, was the structural alteration of the anal gills observed in the treated larvae. While the anal gills of control larvae appeared as sac-like structures covered by smooth cuticle (Figure 3.5A, C), the shrunken anal gills with dark black spots were remarkably found in all treated larvae (Figure 3.5B, D).

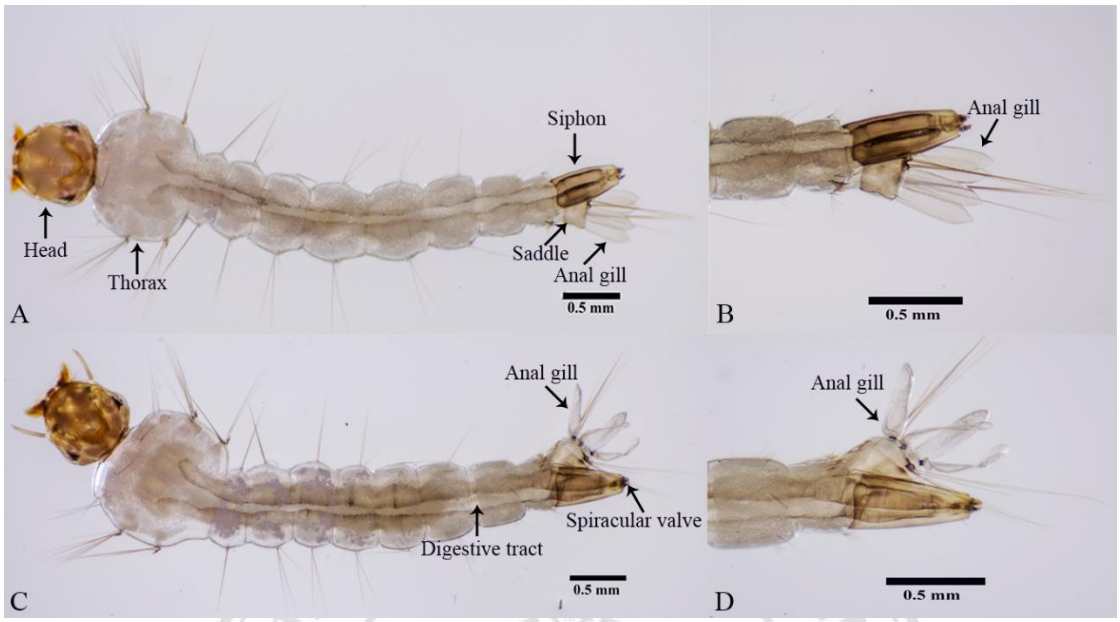


**Figure 3.5** Light micrograph of body segments and anal gills of *Ae. aegypti* larvae. (A, C) Control larvae showing normal body segments (head, thorax, and abdomen), and two pairs of normal gills with sac-like structures covered by smooth cuticle. (B, D) *P. crispum* oil-treated larvae showing normal body regions (head and abdomen), with shrinkage of anal gills.

#### 3.3.2.1.2 Adults

Morphological features of the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti* females treated with *P. crispum* oil were observed and compared with those of the controls under LM. The result revealed that both treated and control adults of three strains showed morphological similarities of the whole body with normal appearance in color, shape, and size (Figure 3.6).

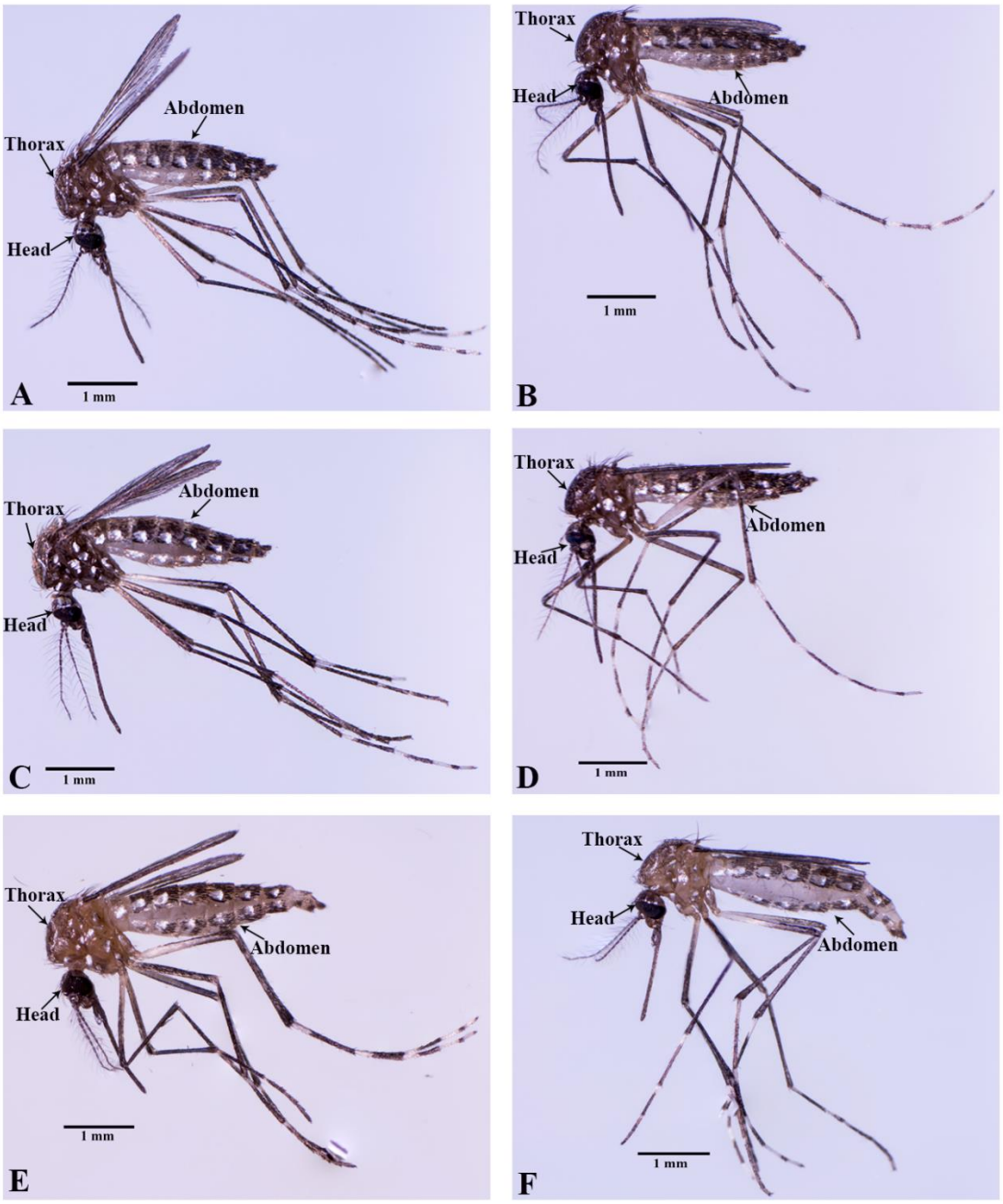




**Figure 3.6** Light micrograph of body segments of *Ae. aegypti* adult females. (A, C, and E) Control adults after dead for 12, 24, and 36 h, respectively. (B, D, and E) *P. crispum* oil-treated adults after dead for 12, 24, and 36 h, respectively.

#### 3.3.2.2 Scanning electron microscopic (SEM) study

##### 3.3.2.2.1 Larvae

Observations on morphological features and cuticular topography of the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti* larvae after treated with *P. crispum* oil were carried out under SEM. The results demonstrated that most organs, except anal gills, in both treated and control larvae had a normal structural appearance (Figure 3.7A, B, G, H). At higher magnification, control larvae showed two pairs of normal gills with sac-like structure covered by smooth cuticle (Figure 3.7C, E), whereas anal gills with shrunken cuticle were found in all treated larvae (Figure 3.7D, F).

##### 3.3.2.2.1 Adults

Morphological observations under SEM of the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti* adults after exposed with *P. crispum* oil for 12, 24, and 36 h revealed that the whole body and other structures in treated and control adults were similarly normal in appearance such as color, shape, and size (Figure 3.8-3.10).

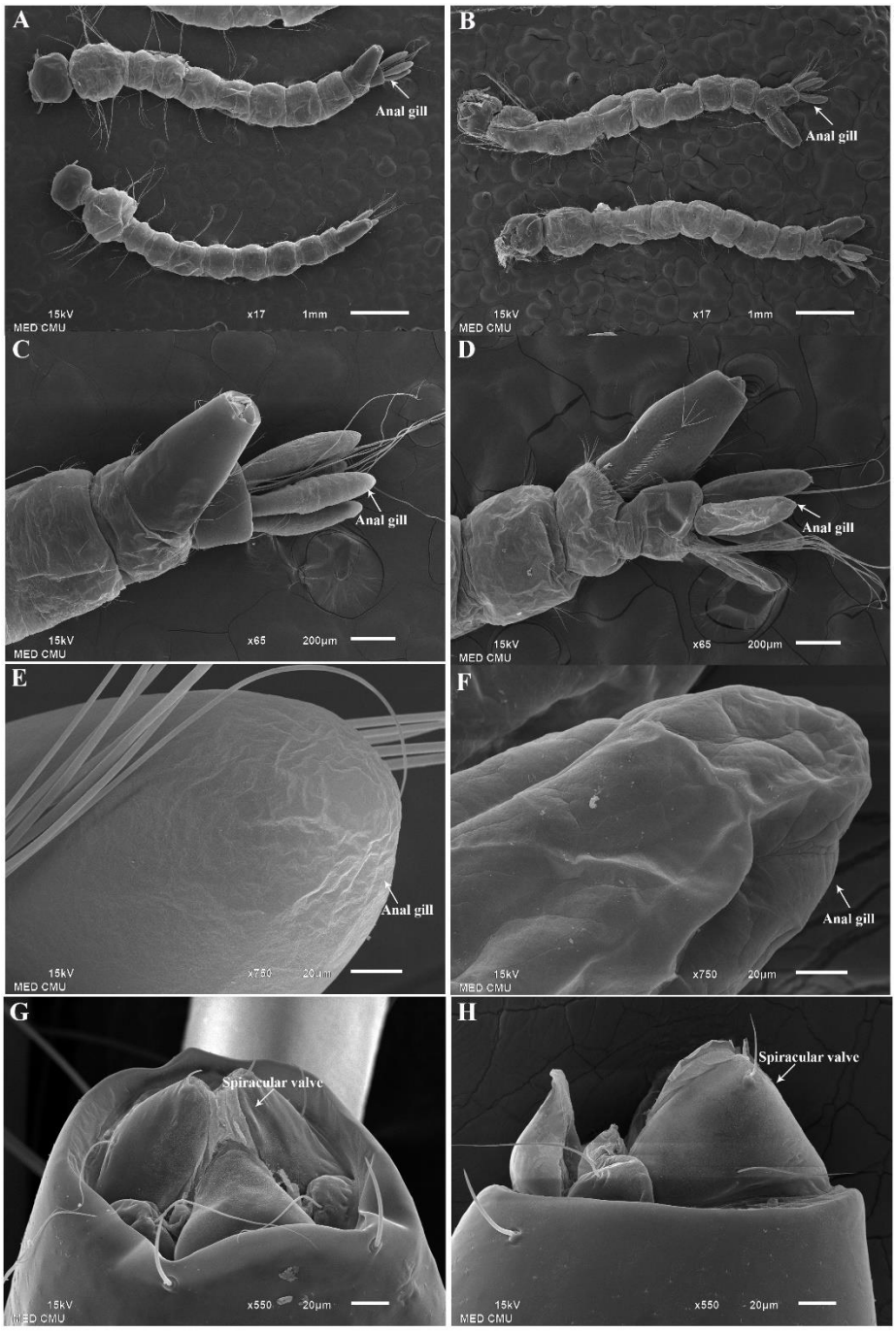


**Figure 3.7** Scanning electron micrograph of body segments and anal gills of *Ae. aegypti* larvae. (A) Control larva showing normal body segments. (B) *P. crispum* oil-treated larvae showing normal body segments with shrunken and collapsed anal gills. (C and E) Control larvae showing cone-shaped normal gills with intact cuticle. (D and F) *P. crispum* oil-treated larvae showing the shrunken and collapsed anal gills. (G and H) Spiracular valve of control and treated larvae showing normal appearance.

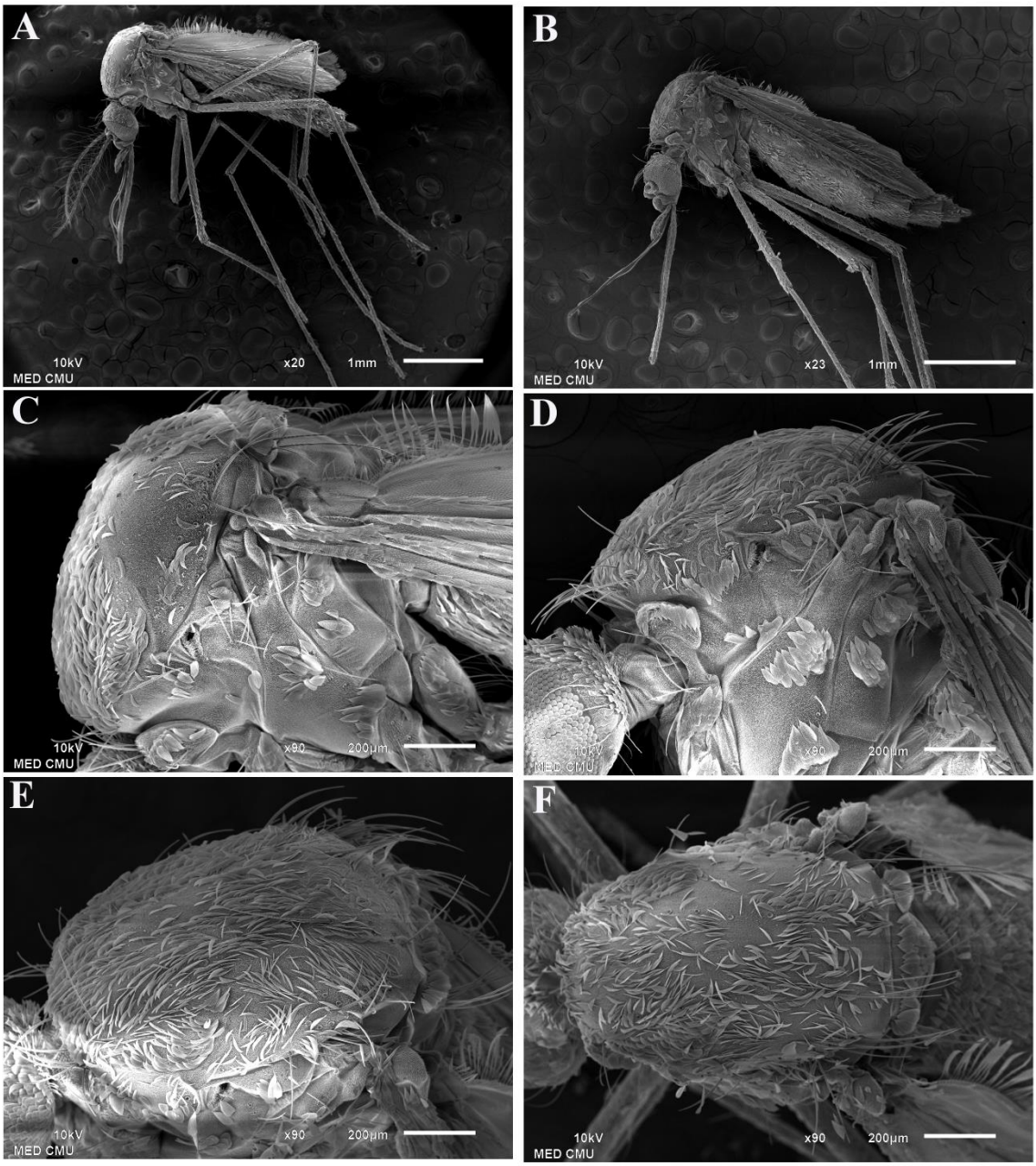




**Figure 3.8** Scanning electron micrograph of body segments of *Ae. aegypti* adults after dead for 12 h showing normal appearance in treated adults with compared to those of control. (A, C, and E) Control adults of whole body segments, thorax in lateral view, and thorax in dorsal view. (B, D, and F) Treated adults of whole body segments, thorax in lateral view, and thorax in dorsal view.

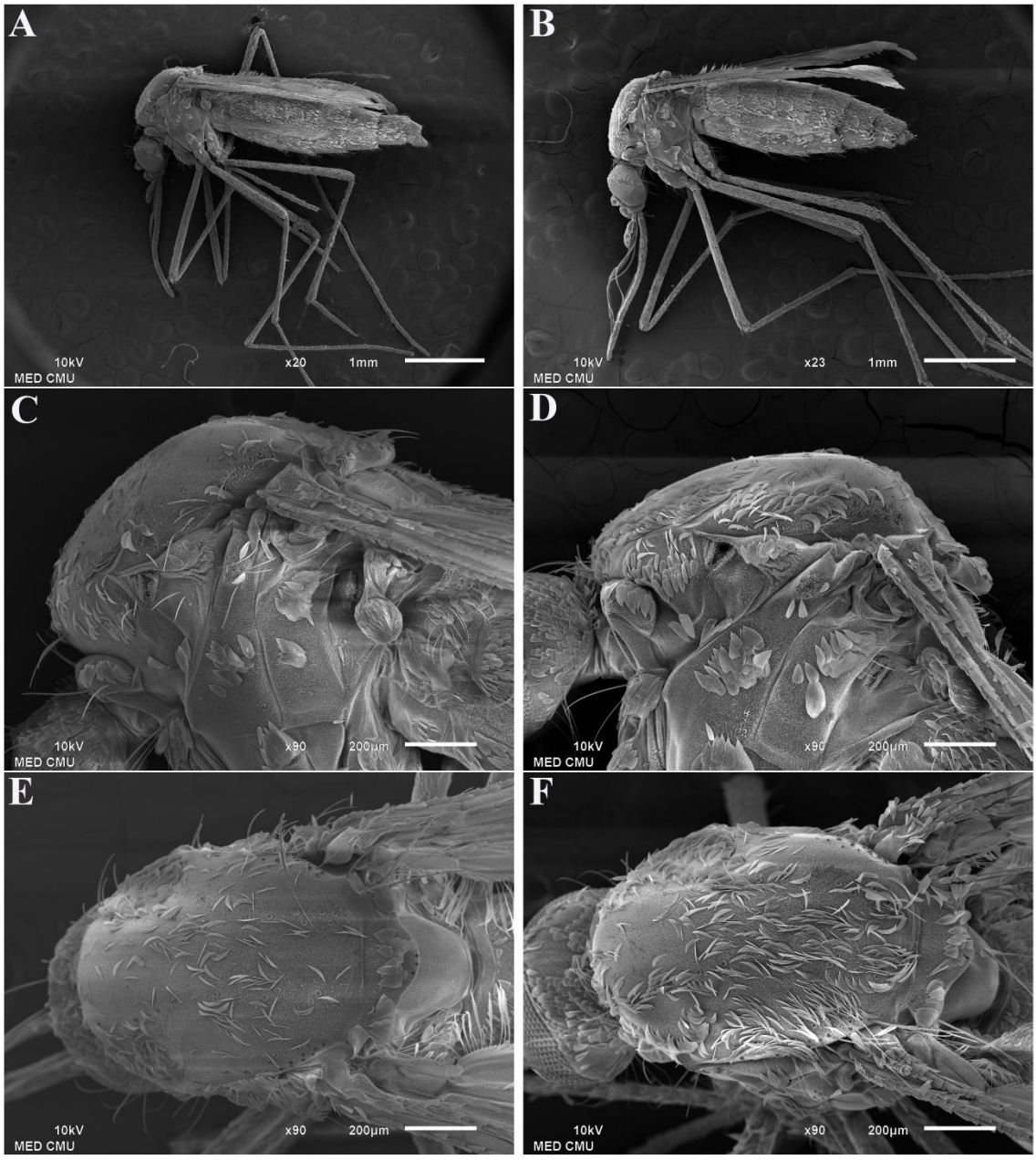




**Figure 3.9** Scanning electron micrograph of body segments of *Ae. aegypti* adults after dead for 24 h showing normal appearance in treated adults with compared to those of control. (A, C, and E) Control adults of whole body segments, thorax in lateral view, and thorax in dorsal view. (B, D, and F) Treated adults of whole body segments, thorax in lateral view, and thorax in dorsal view.

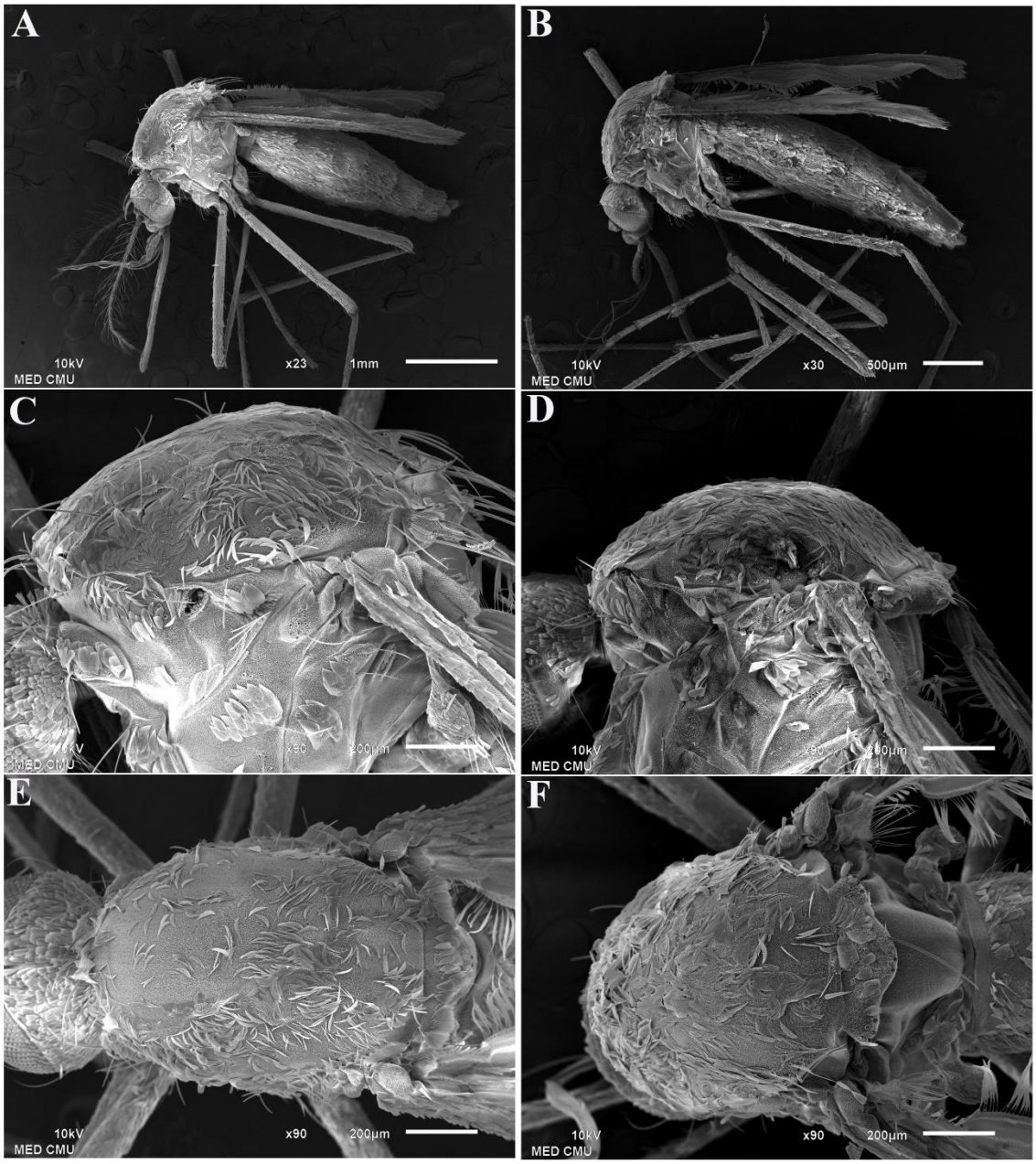




**Figure 3.10** Scanning electron micrograph of body segments of *Ae. aegypti* adults after dead for 36 h showing normal appearance in treated adults with compared to those of control. (A, C, and E) Control adults of whole body segments, thorax in lateral view, and thorax in dorsal view. (B, D, and F) Treated adults of whole body segments, thorax in lateral view, and thorax in dorsal view.

### 3.3.3 Biochemical change observation

#### 3.3.3.1 Biochemical assay on mosquito larvae

##### 3.3.3.1.1 Enzyme activity in untreated larvae (0-h time point)

Investigation of detoxification enzymes in three strains of the untreated larvae revealed that the activities of glutathione-S-transferases (GSTs), *β*-esterases, mixed-function oxidases (MFO), and alkaline phosphatase (ALK) in the UPK-R strain were significantly higher than those observed in the PMD-R and MCM-S strains. While the *α*-esterase activity in UPK-R that relatively comparable to those in PMD-R was statistically higher than those in MCM-S. However, the acid phosphatase (ACP) activity in PMD-R strain was significantly higher than those in UPK-R and MCM-S strains ($P < 0.05$) (Figure 3.11). The level of GSTs activity in UPK-R (0.263 µmol/min/mg protein) was significantly higher than those of PMD-R (0.204 µmol/min/mg protein) and MCM-S (0.107 µmol/min/mg protein). The highest activities of *α*- and *β*-esterases were found in UPK-R (194.67 and 189.76 nmol *α* or *β*-naphthol released/min/mg protein, respectively), followed by PMD-R (181.05 and 152.57 nmol *α* or *β*-naphthol released/min/mg protein, respectively), and MCM-S (131.45 and 118.94 nmol *α* or *β*-naphthol released/min/mg protein, respectively). The activity of MFO enzyme in UPK-R (0.166 nmol cytochrome c equivalent unit/mg protein) was statistically higher than those in PMD-R (0.122 nmol cytochrome c equivalent unit/mg protein) and MCM-S (0.117 nmol cytochrome c equivalent unit/mg protein). The highest activity of ACP was detected in PMD-R, followed by UPK-R and MCM-S with enzyme levels of 97.82, 85.93, and 72.66 nmol *p*-nitrophenol/min/mg protein, respectively. However, the activity of ALK in UPK-R was statistically higher than those in PMD-R and MCM-S, with enzyme levels of 20.99, 12.44, and 13.54 nmol *p*-nitrophenol/min/mg protein, respectively. The levels of acetylcholinesterase (AChE) activity were not significantly different among three larval strains, UPK-R (11.38 nmol ACT hydrolyzed/min/mg protein), PMD-R (10.41 nmol ACT hydrolyzed/min/mg protein), and MCM-S (11.25 nmol ACT hydrolyzed/min/mg protein).

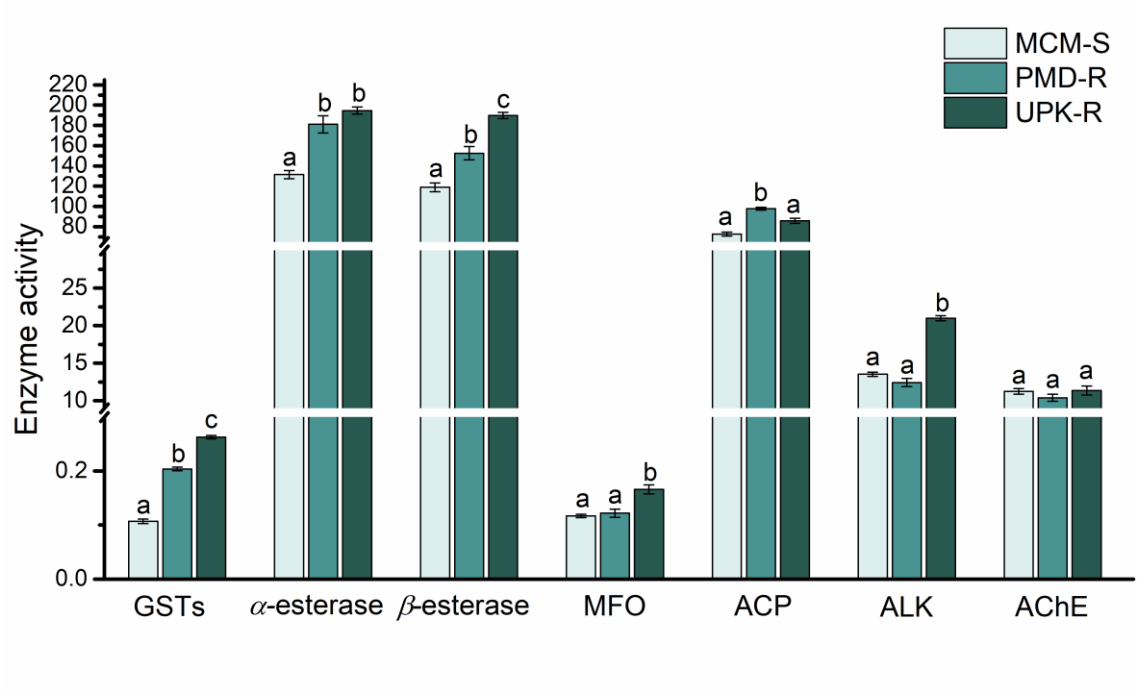


**Figure 3.11** Enzyme activity in the whole body homogenates of the fourth instar larvae pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains (untreated group, 0-h time point). Each bar represents mean±SE of six or ten biological samples from different preparations. Bars with the different letter above indicate statistically significant differences between species (Tukey's HSD test, $P < 0.05$).

#### 3.3.3.1.2 Threshold time for lethal effect of *P. crispum* oil on larvae

Determination of threshold time for lethal effect on the fourth instar larvae of *Ae. aegypti* was carried out by exposure of larvae to *P. crispum* oil with the median lethal concentrations ($LC_{50}$) of 43.22, 44.50, and 44.03 ppm for MCM-S, PMD-R, and UPK-R strains, respectively. It was found that the larval mortality depended on the exposure time. The mortality rate increased with increasing exposure time and approximately 50% larval mortality were recorded after 24 h of exposure. However, there is no significant difference among the mortality rates of three strains of *Ae. aegypti* larvae at 0, 3, 6, 12, and 24 h after treatment (Figure 3.12). The exposure period that provided 20-50% larval mortality was considered as the threshold time for the lethal effect of *P. crispum* oil.

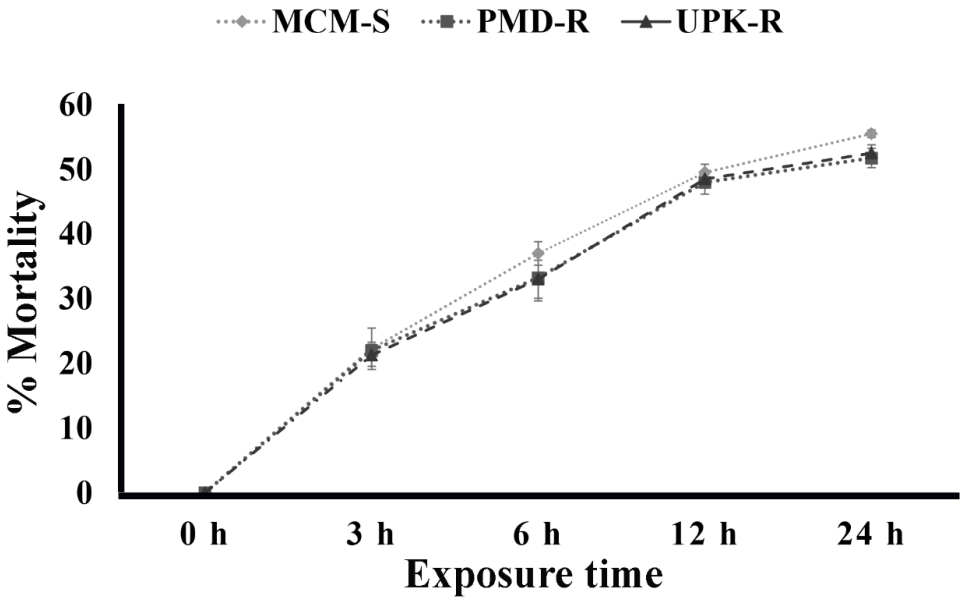


**Figure 3.12** Determination of threshold time for lethal effect of *P. crispum* oil on fourth instar larvae with median lethal concentration ($LC_{50}$) against the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti*. The error bars represent the standard errors of the four replicates.

**3.3.3.1.3 Effects of *P. crispum* oil on biochemical changes in larvae**

The activities of glutathione-S-transferases (GSTs), carboxylesterases ($\alpha$ and $\beta$), mixed-function oxidases (MFO), phosphatases (acid and alkaline), and acethylcholinesterase (AChE) in the control and untreated groups were not significantly different at all-time points. On the other hand, the enzyme activities in the treated group were different from those of the control and untreated groups depending on type of enzyme, strain of mosquito, and time point after treatment.

**3.3.3.1.3.1 GSTs activity**

After exposure to *P. crispum* oil, GSTs activities in MCM-S (3-h time point) and UPK-R (3- and 6-h time points) were significantly increased when compared with those detected in the control groups ($P < 0.05$). After 24 h of treatment GSTs activity levels in MCM-S, PMD-R, and UPK-R were significantly increased by

1.88-fold, 1.83-fold, and 1.86-fold, respectively, over levels in the control. ($P < 0.05$) (Table 3.9 and Figure 3.13).

**Table 3.9** GSTs activity in larvae of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | | GSTs activity (Mean±SE, µmol/min/mg.pro)* | | |
|---|---|---|---|---|
| | | MCM-S | PMD-R | UPK-R |
| 0 hr | | | | |
| | Untreated | 0.107±0.0041[a] | 0.204±0.0035[a] | 0.263±0.0032[a] |
| | Control | 0.110±0.0027[a] | 0.204±0.0035[a] | 0.256±0.0046[a] |
| 3 hr | | | | |
| | Untreated | 0.094±0.0027[a] | 0.203±0.0038[a] | 0.266±0.0040[a] |
| | Control | 0.100±0.0026[a] | 0.203±0.0031[a] | 0.263±0.0059[a] |
| | Treated | 0.140±0.0059[b] | 0.214±0.0068[a] | 0.317±0.0060[b] |
| 6 hr | | | | |
| | Untreated | 0.108±0.0040[a] | 0.219±0.0033[a] | 0.259±0.0037[a] |
| | Control | 0.106±0.0048[a] | 0.211±0.0034[a] | 0.255±0.0076[a] |
| | Treated | 0.115±0.0055[a] | 0.225±0.0055[a] | 0.305±0.0049[b] |
| 24 hr | | | | |
| | Untreated | 0.116±0.0045[a] | 0.227±0.0045[a] | 0.276±0.0063[a] |
| | Control | 0.112±0.0043[a] | 0.242±0.0064[a] | 0.280±0.0072[a] |
| | Treated | 0.210±0.0066[b] | 0.442±0.0207[b] | 0.521±0.0073[b] |

*Means followed by different letters in a column indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$)

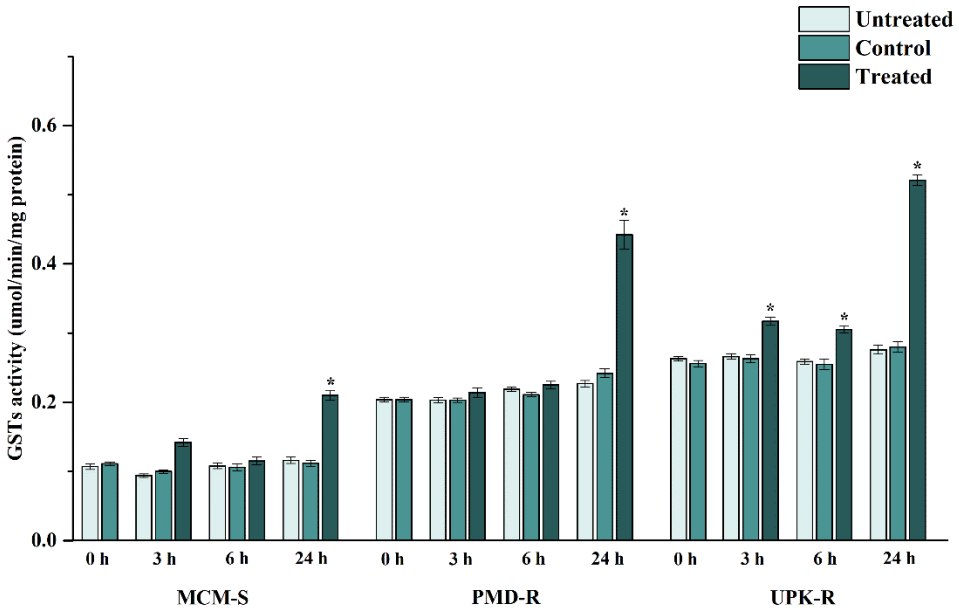


**Figure 3.13** GSTs activity in the whole body homogenates of the fourth instar larvae pyrethroid-susceptible (MCM-S), and resistant (PMD-R and UPK-R) strains exposed to *P. crispum* oil ($LC_{50}$) after 3, 6, and 24 h. Each bar represents mean±SE of six determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

### 3.3.3.1.3.2 Esterases activity

Upon exposure to *P. crispum* oil for 3 h, the levels of *α*- and *β*-esterase activity in UPK-R and MCM-S, respectively, were significantly decreased ($P < 0.05$), whereas the activity of *β*-esterase in UPK-R was increased significantly after 6 h of exposure, when comparing with those observed in the control groups ($P < 0.05$). However, the activity levels of *α*- and *β*-esterases in MCM-S, PMD-R, and UPK-R recorded after 24 h of exposure were significantly increased by 1.6- and 1.6-fold, 1.3- and 1.2-fold, and 1.1- and 1.2-fold, respectively, over those in the controls ($P < 0.05$) (Table 3.10-3.11 and Figure 3.14-3.15).

**Table 3.10** $\alpha$-Esterase activity in larvae of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | $\alpha$-Esterase activity (Mean±SE, nmol/min/mg.pro)* | | |
|---|---|---|---|
| | MCM-S | PMD-R | UPK-R |
| 0 hr | | | |
| Untreated | 131.45±4.12[a] | 181.05±8.49[a] | 194.67±3.48[a] |
| Control | 124.00±6.21[a] | 186.59±5.19[a] | 195.78±6.80[a] |
| 3 hr | | | |
| Untreated | 131.68±6.66[a] | 194.94±4.77[a] | 207.48±4.89[a] |
| Control | 139.46±5.70[a] | 190.79±4.85[a] | 207.83±4.71[a] |
| Treated | 124.14±5.77[a] | 182.92±3.57[a] | 167.04±3.53[b] |
| 6 hr | | | |
| Untreated | 136.18±6.08[a] | 193.42±6.64[a] | 205.57±4.34[a] |
| Control | 134.82±4.18[a] | 194.21±4.72[a] | 209.94±4.91[a] |
| Treated | 128.14±5.31[a] | 196.83±7.07[a] | 211.47±4.86[a] |
| 24 hr | | | |
| Untreated | 133.10±3.52[a] | 178.64±5.35[a] | 192.88±3.79[a] |
| Control | 125.92±5.56[a] | 174.59±3.40[a] | 193.33±4.40[a] |
| Treated | 202.19±5.08[b] | 219.44±6.71[b] | 215.14±5.35[b] |

*Means followed by different letters in a column indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$)

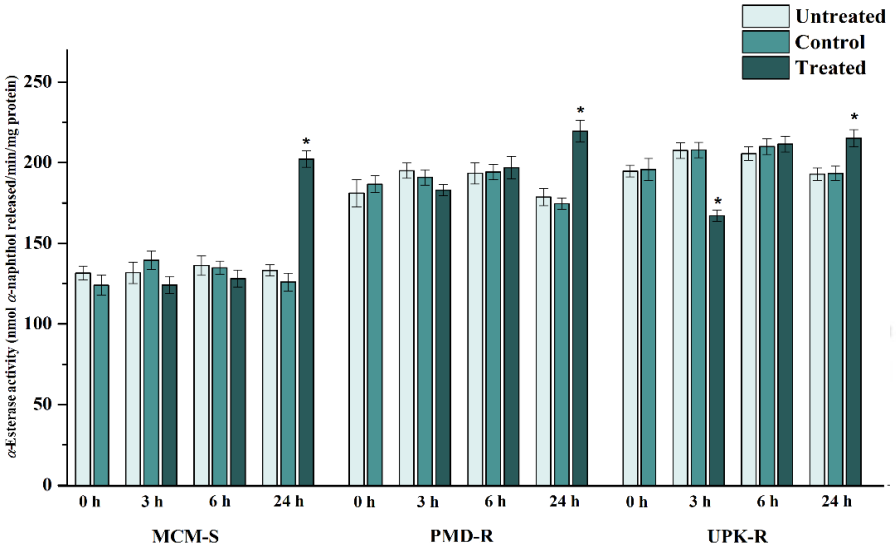


**Figure 3.14** $\alpha$-Esterase activity in the whole body homogenates of the fourth instar larvae pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains exposed to *P. crispum* oil ($LC_{50}$) after 3, 6, and 24 h. Each bar represents mean±SE of ten determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

**Table 3.11** $\beta$-Esterase activity in larvae of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | $\beta$-Esterase activity (Mean±SE, nmol/min/mg.pro)* | | |
|---|---|---|---|
| | MCM-S | PMD-R | UPK-R |
| 0 hr | | | |
| Untreated | 118.94±4.33[a] | 152.57±6.60[a] | 189.76±3.02[a] |
| Control | 123.19±6.41[a] | 160.76±4.87[a] | 187.13±6.23[a] |
| 3 hr | | | |
| Untreated | 132.06±6.14[a] | 156.01±4.94[a] | 175.71±3.55[a] |
| Control | 136.83±5.59[a] | 159.74±4.41[a] | 177.34±4.41[a] |
| Treated | 112.93±4.70[b] | 165.00±2.46[a] | 166.28±5.19[a] |
| 6 hr | | | |
| Untreated | 122.85±5.78[a] | 159.05±5.81[a] | 182.88±4.86[a] |
| Control | 123.36±4.74[a] | 160.36±3.90[a] | 180.70±4.18[a] |
| Treated | 117.39±3.38[a] | 173.03±6.48[a] | 197.61±3.70[b] |
| 24 hr | | | |
| Untreated | 128.42±4.33[a] | 158.87±6.04[a] | 170.79±4.15[a] |
| Control | 125.28±4.16[a] | 157.25±2.83[a] | 169.66±2.92[a] |
| Treated | 203.34±8.31[b] | 185.03±7.41[b] | 212.09±5.99[b] |

*Means followed by different letters indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$)

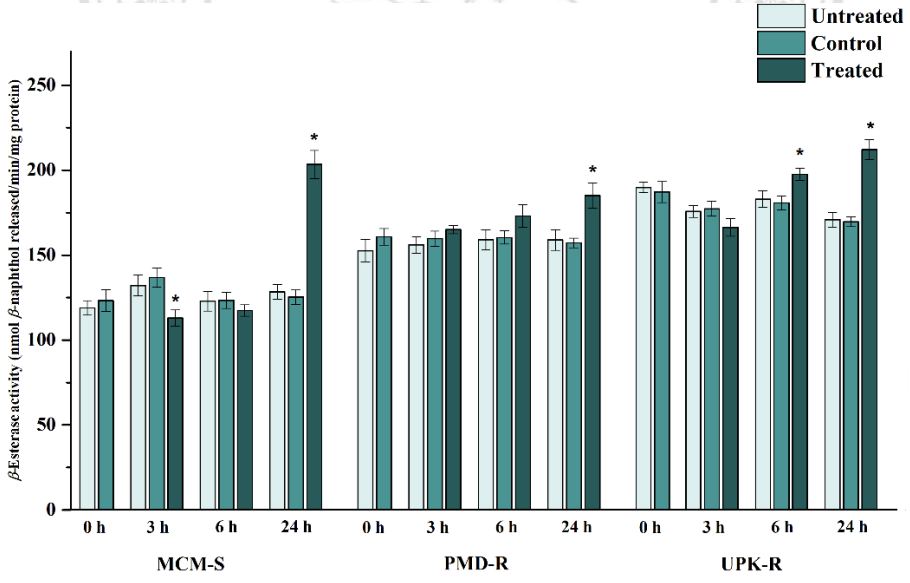


**Figure 3.15** $\beta$-Esterase activity in the whole body homogenates of the fourth instar larvae pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains exposed to *P. crispum* oil ($LC_{50}$) after 3, 6, and 24 h. Each bar represents mean±SE of ten determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

#### 3.3.3.1.3.3 MFO activity

The activity levels of MFO in three groups of MCM-S, PMD-R, and UPK-R larvae are presented in Table 3.12 and Figure 3.16. It was found that MFO activities in the control and untreated larval groups were slightly decreased with increasing exposure time (0 to 24 h) in all strains of mosquitoes. The MFO activity in UPK-R was statistically higher than those in the PMD-R and MCM-S in all larval groups at every time point. However, there was no significant difference of MFO activity in *P. crispum* oil-treated larvae of all strains when comparing with those recorded in the controls, except for those in UPK-R (0.093 nmol cytochrome c equivalent unit/mg protein) recorded after 24 h of treatment ($P < 0.05$).

**Table 3.12** MFO activity in larvae of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | MFO activity (Mean±SE, nmol/min/mg.pro)* | | |
|---|---|---|---|
| | MCM-S | PMD-R | UPK-R |
| 0 hr | | | |
| Untreated | 0.117±0.0032[a] | 0.122±0.0077[a] | 0.166±0.0084[a] |
| Control | 0.106±0.0046[a] | 0.126±0.0085[a] | 0.151±0.0065[a] |
| 3 hr | | | |
| Untreated | 0.106±0.0057[a] | 0.102±0.0063[a] | 0.135±0.0050[a] |
| Control | 0.101±0.0045[a] | 0.104±0.0071[a] | 0.138±0.0059[a] |
| Treated | 0.098±0.0039[a] | 0.105±0.0078[a] | 0.129±0.0056[a] |
| 6 hr | | | |
| Untreated | 0.095±0.0034[a] | 0.093±0.0044[a] | 0.129±0.0040[a] |
| Control | 0.098±0.0045[a] | 0.091±0.0063[a] | 0.136±0.0074[a] |
| Treated | 0.089±0.0047[a] | 0.097±0.0067[a] | 0.125±0.0067[a] |
| 24 hr | | | |
| Untreated | 0.077±0.0045[a] | 0.075±0.0057[a] | 0.115±0.0042[a] |
| Control | 0.080±0.0031[a] | 0.076±0.0043[a] | 0.119±0.0077[a] |
| Treated | 0.073±0.0035[a] | 0.066±0.0045[a] | 0.093±0.0043[b] |

*Means followed by different letters in a column indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$)

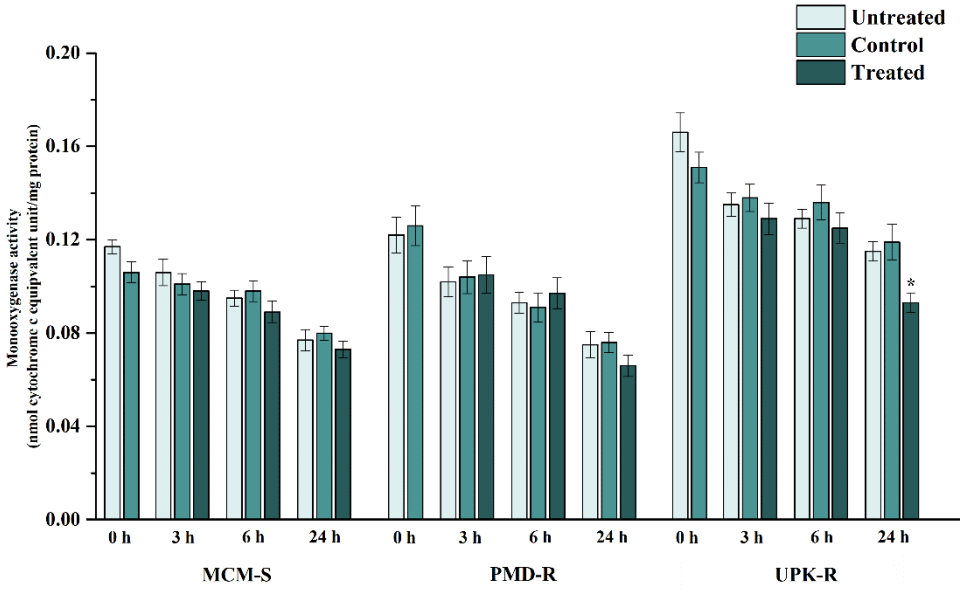


**Figure 3.16** MFO activity in the whole body homogenates of the fourth instar larvae pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains exposed to *P. crispum* oil ($LC_{50}$) after 3, 6, and 24 h. Each bar represents mean±SE of ten determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

### 3.3.3.1.3.4 ACP and ALK activity

The activities of ACP in the control and untreated larval groups were slightly increased with increasing exposure time (0 to 24 h) in all strains of mosquitoes (Table 3.13 and Figure 3.17), whereas those of ALK was decreased (Table 3.14 and Figure 3.18). In *P. crispum* oil-treated larvae, the activity of ACP was significantly increased after exposure for 3 h (PMD-R strain) and 6 h (MCM-S, PMD-R, and UPK-R strains). The significant increase of ALK activity was detected in MCM-S (3- and 6-h time points) and UPK-R (6-h time point). Correspondingly, the activity levels of both ACP and ALK in MCM-S (16 and 112%, respectively), PMD-R (34 and 100%, respectively), and UPK-R (6 and 63%, respectively) were significantly increased after 24 h of exposure, when compared with those in the controls ($P < 0.05$).

**Table 3.13** ACP activity in larvae of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | ACP activity (Mean±SE, nmol *p*-nitrophenol/min/mg.pro)* | | |
|---|---|---|---|
| | MCM-S | PMD-R | UPK-R |
| 0 hr | | | |
| Untreated | 72.66±1.9555[a] | 97.82±1.4936[a] | 85.93±2.5277[a] |
| Control | 69.95±1.8168[a] | 94.60±1.8733[a] | 80.66±1.6317[a] |
| 3 hr | | | |
| Untreated | 91.02±3.0743[a] | 109.47±1.5163[a] | 97.10±2.8551[a] |
| Control | 87.66±2.9277[a] | 106.08±2.0181[a] | 93.76±2.6460[a] |
| Treated | 83.65±2.5826[a] | 125.05±1.9284[b] | 83.38±2.0397[b] |
| 6 hr | | | |
| Untreated | 77.99±2.9507[a] | 109.60±2.0875[a] | 86.76±2.2992[a] |
| Control | 81.75±2.9215[a] | 102.82±1.6270[a] | 86.85±1.8752[a] |
| Treated | 95.85±4.5353[b] | 133.74±2.6227[b] | 95.41±1.6366[b] |
| 24 hr | | | |
| Untreated | 100.02±4.8075[a] | 125.55±2.0694[a] | 101.57±3.1404[a] |
| Control | 104.56±4.1956[a] | 126.39±1.9456[a] | 103.13±1.6031[a] |
| Treated | 121.12±2.5162[b] | 168.97±2.8467[b] | 109.35±2.4132[b] |

*Means followed by different letters in a column indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$)

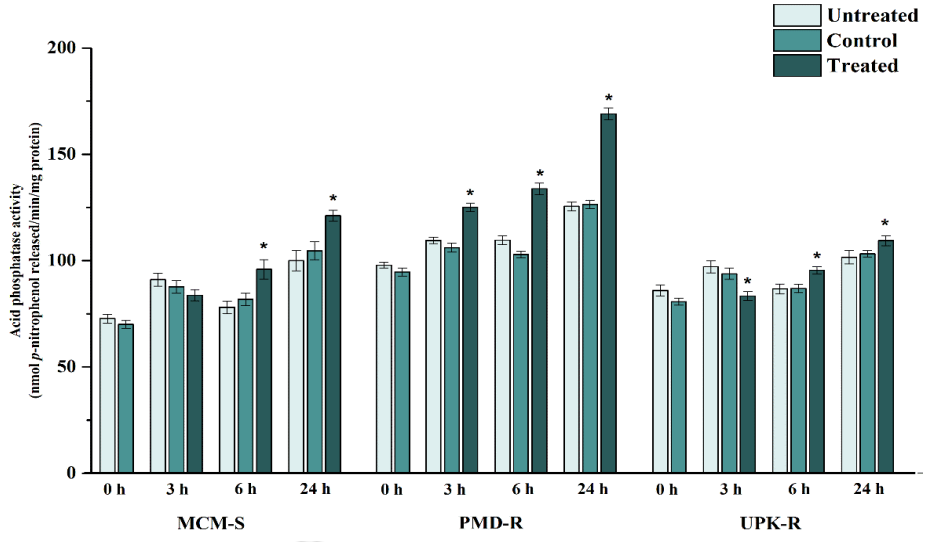


**Figure 3.17** ACP activity in the whole body homogenates of the fourth instar larvae pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains exposed to *P. crispum* oil ($LC_{50}$) after 3, 6, and 24 h. Each bar represents mean±SE of ten determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

**Table 3.14** ALK activity in larvae of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | ALK activity (Mean±SE, nmol *p*-nitrophenol/min/mg.pro)* | | |
|---|---|---|---|
| | MCM-S | PMD-R | UPK-R |
| 0 hr | | | |
| Untreated | 13.54±0.2842[a] | 12.44±0.5494[a] | 20.99±0.3529[a] |
| Control | 12.88±0.4900[a] | 12.85±0.9377[a] | 20.66±0.7266[a] |
| 3 hr | | | |
| Untreated | 10.10±0.2454[a] | 10.93±0.4941[a] | 20.49±0.4341[a] |
| Control | 9.81±0.4696[a] | 10.91±0.5234[a] | 19.59±0.9677[a] |
| Treated | 12.52±0.4095[b] | 10.73±0.9040[a] | 19.08±0.4344[a] |
| 6 hr | | | |
| Untreated | 8.85±0.3989[a] | 9.63±0.3433[a] | 16.67±0.4777[a] |
| Control | 9.17±0.1736[a] | 10.71±0.6195[a,b] | 15.62±0.4466[a] |
| Treated | 11.74±0.1937[b] | 11.13±0.2611[b] | 20.40±0.6988[b] |
| 24 hr | | | |
| Untreated | 7.24±0.3285[a] | 6.48±0.3493[a] | 9.44±0.3603[a] |
| Control | 7.33±0.2255[a] | 6.90±0.3074[a] | 10.38±0.2502[a] |
| Treated | 15.54±0.5609[b] | 13.03±0.5455[b] | 16.93±0.6474[b] |

*Means followed by different letters in a column indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$)

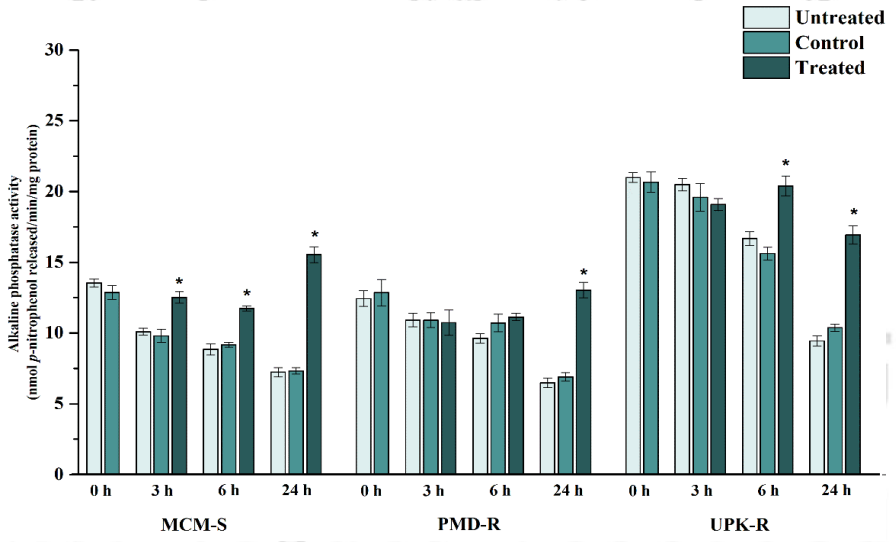


**Figure 3.18** ALK activity in the whole body homogenates of the fourth instar larvae pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains exposed to *P. crispum* oil ($LC_{50}$) after 3, 6, and 24 h. Each bar represents mean±SE of ten determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

#### 3.3.3.1.3.5 AChE activity

The effects of *P. crispum* oil on AChE activity in MCM-S, PMD-R, and UPK-R strains are shown in Table 3.15 and Figure 3.19. Except for the treated MCM-S at 3-h time point, AChE activities in all larval strains recorded at 3- and 6-h time points were significantly decreased when compared with those of the controls. However, after 24 h of treatment, the activity levels of AChE in all strains were significantly decreased ($P < 0.05$).

**Table 3.15** AChE activity in larvae of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | AChE activity (Mean±SE, nmol/min/mg.pro)* | | |
|---|---|---|---|
| | MCM-S | PMD-R | UPK-R |
| 0 hr | | | |
| Untreated | 11.25±0.385[a] | 10.41±0.469[a] | 11.38±0.602[a] |
| Control | 10.67±0.295[a] | 10.61±0.461[a] | 11.42±0.423[a] |
| 3 hr | | | |
| Untreated | 9.72±0.259[a] | 10.81±0.556[a] | 14.90±0.582[a] |
| Control | 10.16±0.459[a] | 10.37±0.373[a] | 14.38±0.757[a] |
| Treated | 9.61±0.315[a] | 8.26±0.310[b] | 12.09±0.490[b] |
| 6 hr | | | |
| Untreated | 10.33±0.287[a] | 9.88±0.382[a,b] | 13.22±0.384[a] |
| Control | 10.67±0.471[a] | 10.16±0.476[a] | 13.90±0.548[a] |
| Treated | 8.47±0.309[b] | 8.66±0.331[b] | 10.58±0.278[b] |
| 24 hr | | | |
| Untreated | 12.81±0.415[a] | 12.12±0.604[a] | 15.06±0.456[a] |
| Control | 12.90±0.460[a] | 11.90±0.441[a] | 15.27±0.350[a] |
| Treated | 8.65±0.316[b] | 8.67±0.366[b] | 12.93±0.352[b] |

*Means followed by different letters in a column indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$)

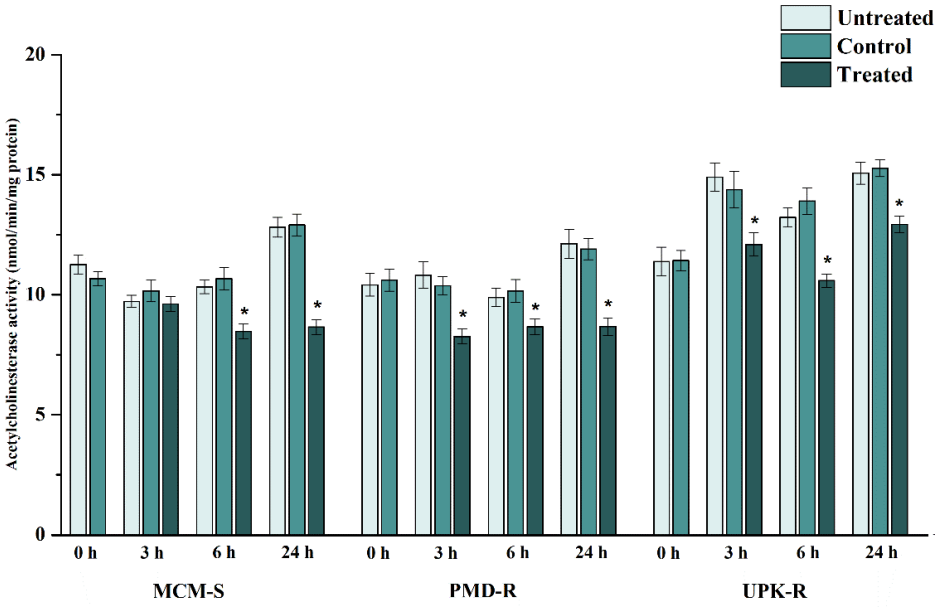


**Figure 3.19** AChE activity in the whole body homogenates of the fourth instar larvae pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains exposed to *P. crispum* oil ($LC_{50}$) after 3, 6, and 24 h. Each bar represents mean±SE of ten determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

### 3.3.3.2 Biochemical assay on mosquito adults

#### 3.3.3.2.1 Enzyme activity in untreated adults (0-h time point)

Evaluation on detoxifying enzymes in three strains of untreated adults demonstrated that the activities of GSTs, $\alpha$- and $\beta$-esterases, MFO, and ACP in UPK-R were higher than those in PMD-R and MCM-S. Nevertheless, the level of ALK activity in PMD-R that relatively comparable to those in MCM-S was statistically higher than those in UPK-R (Figure 3.20). The significant level of GSTs activity was observed in UPK-R (0.419 µmol/min/mg protein), followed by PMD-R (0.394 µmol/min/mg protein), and MCM-S (0.305 µmol/min/mg protein). The highest activities of $\alpha$- and $\beta$-esterases were recorded in UPK-R (152.97 and 134.82 nmol $\alpha$ or $\beta$-naphthol released/min/mg protein), followed by MCM-S (120.08 and 110.54 nmol $\alpha$ or $\beta$-naphthol released/min/mg protein), and PMD-R (112.70 and 100.36 nmol $\alpha$ or $\beta$-naphthol

released/min/mg protein). The activities of MFO enzyme in UPK-R were statistically higher than those in PMD-R and MCM-S, with enzyme levels of 1.713, 1.268, and 0.903 nmol cytochrome c equivalent unit/mg protein, respectively. The highest activity of ACP was detected in UPK-R, followed by MCM-S and PMD-R with enzyme levels of 155.67, 135.65, and 109.69 nmol *p*-nitrophenol/min/mg protein, respectively. Conversely, the highest level of ALK was observed in PMD-R, followed by MCM-S, and UPK-R, with enzyme levels of 12.14, 11.37, and 9.46 nmol *p*-nitrophenol/min/mg protein, respectively. AChE activity in UPK-R was significantly higher than those in MCM-S and PMD-R, with enzyme levels of 11.80, 10.71, and 10.07 nmol ACT hydrolyzed/min/mg protein, respectively.

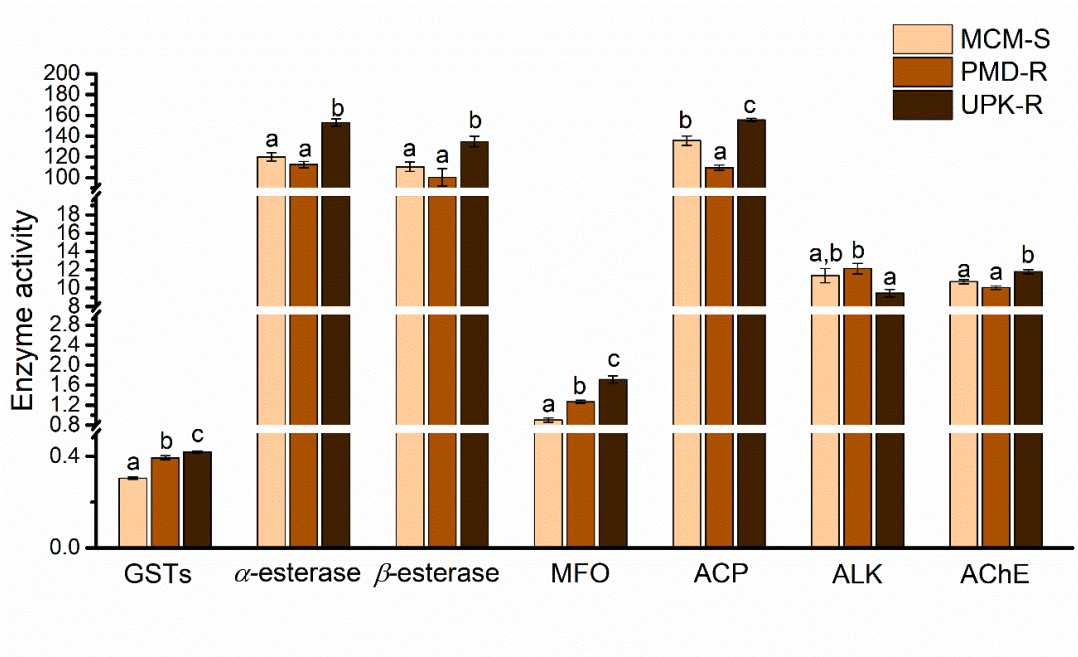


**Figure 3.20** Enzyme activity in the whole body homogenates of the adults pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains (untreated group, 0-h time point). Each bar represents mean ± SE of six or ten biological samples from different preparations. Bars with the different letter above indicate statistically significant differences between species (Tukey's HSD test, $P < 0.05$).

#### 3.3.3.2.2 Threshold time for lethal effect of *P. crispum* oil on adults

Determination of threshold time for lethal effect on adult females of *Ae. aegypti* was performed by exposing to *P. crispum* oil, with $LD_{50}$ of 6.01, 6.15, and 6.12 µg/mg female for MCM-S, PMD-R, and UPK-R, respectively. It was found that the adult mortality depended on the exposure time. The mortality rate increased with increasing exposure time and approximately 50 and 60% mortalities were recorded after 24 and 48 h of exposure. However, there is no significant difference among the mortality rates of three strains of *Ae. aegypti* adults at 0, 3, 6, 12, 24, and 48 h after treatment (Figure 3.21). The exposure period that provided 20-60% adult mortality was considered as the threshold time for the lethal effect of *P. crispum* oil.

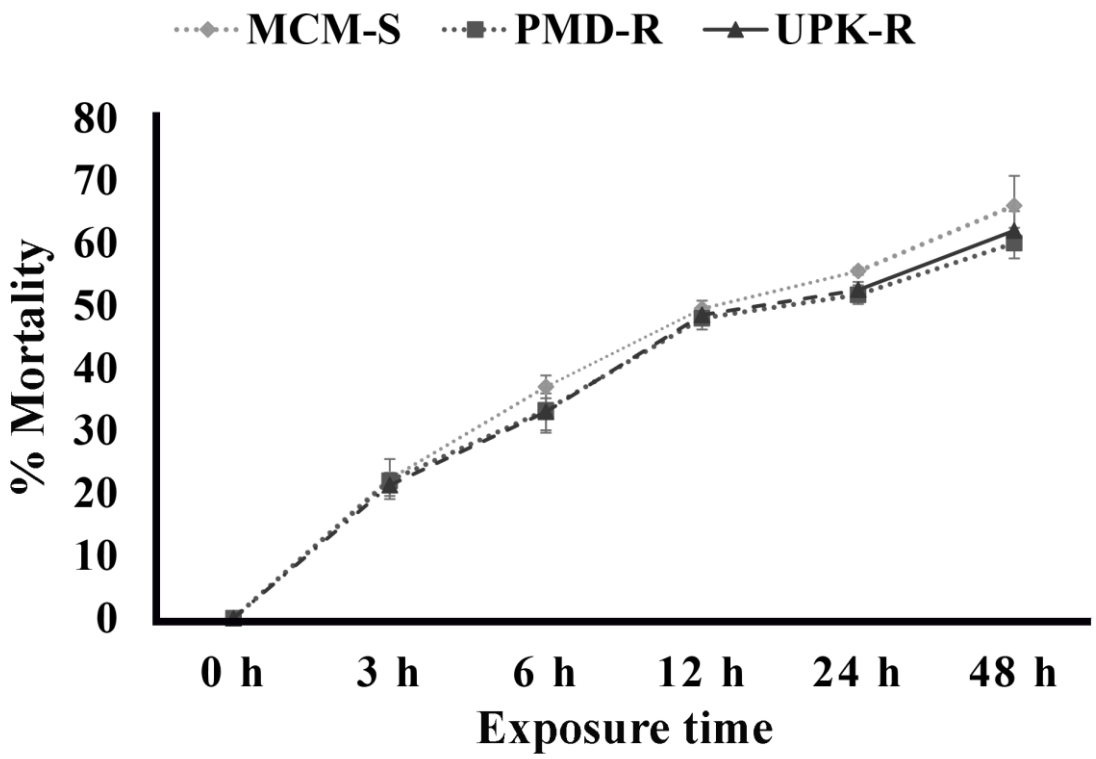


**Figure 3.21** Determination of threshold time for lethal effect of *P. crispum* oil on adults with median lethal dose ($LD_{50}$) against the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Ae. aegypti*. The error bars represents the standard errors of the four replicates.

#### 3.3.3.2.3 Effects of *P. crispum* oil on biochemical changes in adults

The activities of GSTs, $\alpha$- and $\beta$-esterases, MFO, ACP and ALK, and AChE in the control and untreated groups were not significantly different at all-time points. By contrast, the enzyme activities in the treated group were different from those of the control and untreated groups depending on type of enzyme, strain of mosquito, and time point after treatment.

##### 3.3.3.2.3.1 GSTs activity

After exposure to *P. crispum* oil, the GSTs activity levels in three groups of MCM-S, PMD-R, and UPK-R adults were not significantly different at all-time points (Table 3.16 and Figure 3.22).

**Table 3.16** GSTs activity in adults of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | GSTs activity (Mean±SE, µmol/min/mg.pro)* | | |
|---|---|---|---|
| | MCM-S | PMD-R | UPK-R |
| 0 hr | | | |
| Untreated | 0.305±0.005[a] | 0.394±0.009[a] | 0.419±0.005[a] |
| Control | 0.290±0.007[a] | 0.388±0.011[a] | 0.417±0.008[a] |
| 24 hr | | | |
| Untreated | 0.275±0.009[a] | 0.367±0.005[a] | 0.384±0.006[a] |
| Control | 0.270±0.005[a] | 0.354±0.008[a] | 0.386±0.004[a] |
| Treated | 0.274±0.006[a] | 0.376±0.009[a] | 0.395±0.006[a] |
| 48 hr | | | |
| Untreated | 0.242±0.004[a] | 0.343±0.008[a] | 0.376±0.006[a] |
| Control | 0.241±0.003[a] | 0.325±0.007[a] | 0.364±0.006[a] |
| Treated | 0.247±0.004[a] | 0.328±0.009[a] | 0.376±0.008[a] |

*Means followed by different letters in a column indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$)

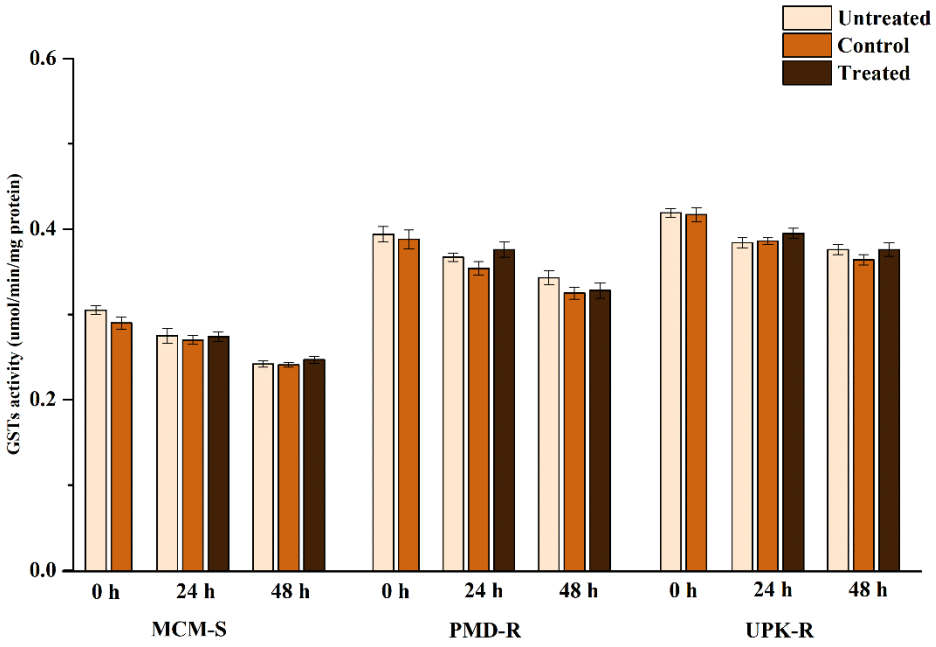


**Figure 3.22** GSTs activity in the whole body homogenates of the adult pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains exposed to *P. crispum* oil ($LD_{50}$) after 24 and 48 h. Each bar represents mean±SE of six determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

#### 3.3.3.2.3.2 Esterases activity

Upon exposure to *P. crispum* oil for 24 h, the activities of *α*-esterase in the adults of MCM-S, PMD-R, and UPK-R were insignificantly increased as comparing to those of the controls, whereas the activity level of this enzyme in these mosquitoes were either insignificantly increased or decreased after exposure with *P. crispum* oil for 48 h (Table 3.17 and Figure 3.23). However, when comparing with the control groups, the levels of *β*-esterase activity in MCM-S and UPK-R adults were significantly increased after 24 h of exposure to *P. crispum* oil ($P < 0.05$), whereas that of PMD-R was significantly increased after 48 h of treatment ($P < 0.05$) (Table 3.18 and Figure 3.24).

**Table 3.17** $\alpha$-Esterase activity in adults of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | $\alpha$-Esterase activity (Mean±SE, nmol/min/mg.pro)* | | |
|---|---|---|---|
| | MCM-S | PMD-R | UPK-R |
| 0 hr | | | |
| Untreated | 120.08±3.94[a] | 112.70±3.05[a] | 152.97±3.65[a] |
| Control | 116.68±3.48[a] | 122.52±2.72[a] | 153.54±6.08[a] |
| 24 hr | | | |
| Untreated | 107.59±5.87[a] | 134.68±8.72[a] | 136.11±5.27[a] |
| Control | 108.11±2.98[a] | 135.97±8.30[a] | 136.06±8.99[a] |
| Treated | 125.39±6.28[a] | 141.24±9.72[a] | 145.07±8.35[a] |
| 48 hr | | | |
| Untreated | 107.09±4.05[a] | 111.42±7.92[a] | 124.47±5.74[a] |
| Control | 107.18±4.46[a] | 118.12±9.30[a] | 128.27±5.03[a] |
| Treated | 114.65±5.17[a] | 117.45±6.73[a] | 116.42±2.69[a] |

*Means followed by different letters in a column indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$

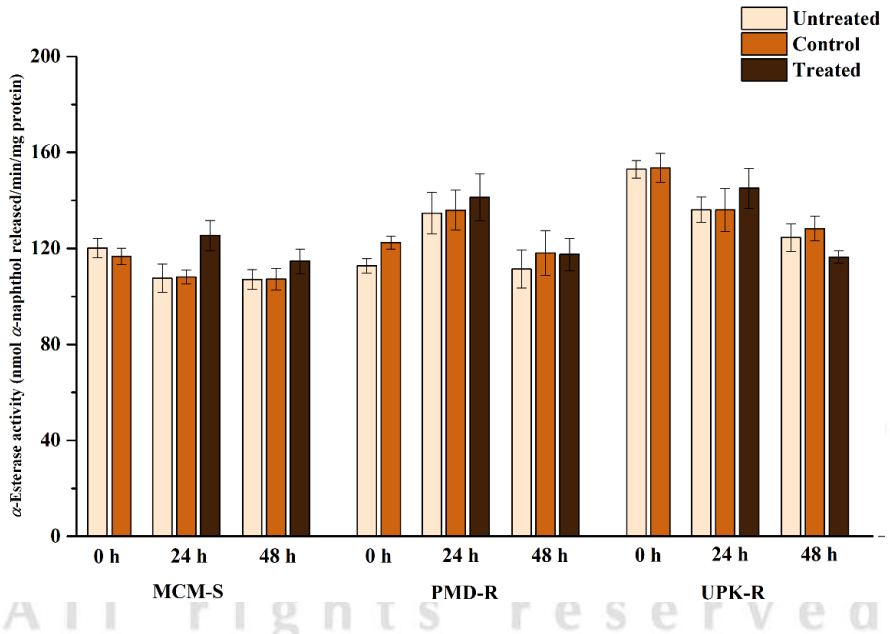




**Figure 3.23** $\alpha$-Esterase activity in the whole body homogenates of the adult pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains exposed to *P. crispum* oil ($LD_{50}$) after 24 and 48 h. Each bar represents mean±SE of ten determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

**Table 3.18** *β*-Esterase activity in adults of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | *β*-Esterase activity (Mean±SE, nmol/min/mg.pro)* | | |
|---|---|---|---|
| | MCM-S | PMD-R | UPK-R |
| 0 hr | | | |
| Untreated | 110.54±4.53[a] | 100.36±8.29[a] | 134.82±5.05[a] |
| Control | 108.55±2.34[a] | 99.61±3.79[a] | 131.89±6.17[a] |
| 24 hr | | | |
| Untreated | 117.74±4.80[a] | 104.94±8.02[a] | 124.40±4.27[a] |
| Control | 116.82±4.78[a] | 114.68±4.07[a] | 125.91±5.03[a] |
| Treated | 149.62±6.25[b] | 117.93±2.75[a] | 142.82±4.54[b] |
| 48 hr | | | |
| Untreated | 117.93±5.50[a] | 95.66±3.63[a] | 123.71±4.76[a] |
| Control | 119.44±6.74[a] | 88.98±4.92[a] | 127.44±8.11[a] |
| Treated | 139.72±7.01[a] | 112.88±2.97[b] | 127.18±3.78[a] |

*Means followed by different letters in a column indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$

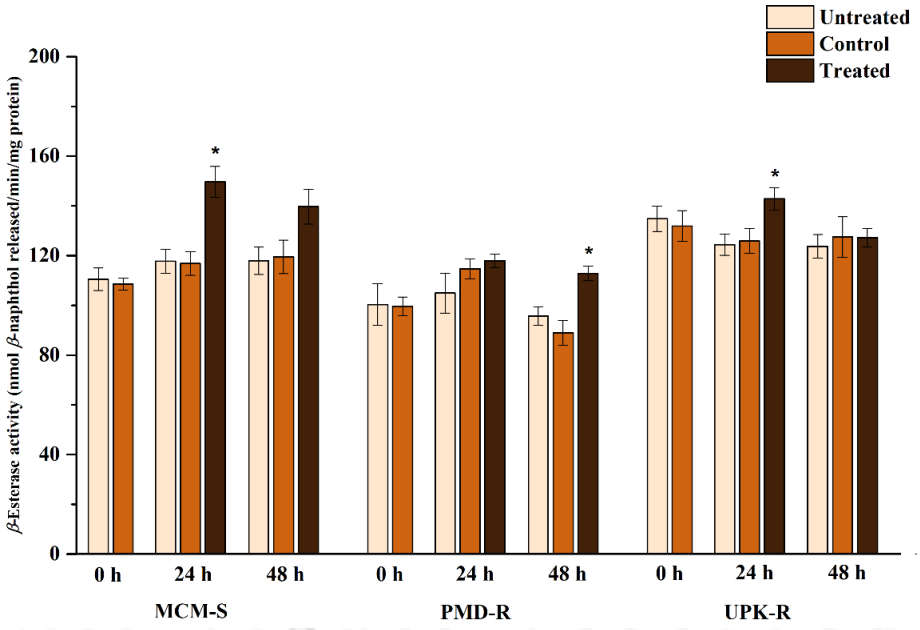


**Figure 3.24** *β*-Esterase activity in the whole body homogenates of the adults pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains exposed to *P. crispum* oil ($LD_{50}$) after 24 and 48 h. Each bar represents mean±SE of ten determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

#### 3.3.3.2.3.3 MFO activity

The activity levels of MFO in three groups of MCM-S, PMD-R, and UPK-R adults are presented in Table 3.19 and Figure 3.25. When comparing with the controls, the MFO activity in *P. crispum* oil-treated MCM-S adults was significantly increased after 24 and 48 h of exposures ($P < 0.05$). The level of MFO activity in treated PMD-R adults was significantly increased after 24 h of treatment ($P < 0.05$), whereas that this enzyme activity in the treated UPK-R was not significantly different from that of the controls at all-time points.

**Table 3.19** MFO activity in adults of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | MFO activity (Mean±SE, nmol cytochrome c equivalent unit/mg.pro)* | | |
|---|---|---|---|
| | MCM-S | PMD-R | UPK-R |
| 0 hr | | | |
| Untreated | 0.903±0.043[a] | 1.268±0.029[a] | 1.713±0.072[a] |
| Control | 0.997±0.062[a] | 1.309±0.032[a] | 1.782±0.091[a] |
| 24 hr | | | |
| Untreated | 1.060±0.059[a,b] | 1.355±0.021[a,b] | 2.105±0.125[a] |
| Control | 1.028±0.064[a] | 1.318±0.026[a] | 2.061±0.102[a] |
| Treated | 1.219±0.039[b] | 1.411±0.033[b] | 2.012±0.121[a] |
| 48 hr | | | |
| Untreated | 1.069±0.051[a,b] | 1.368±0.026[a] | 2.092±0.080[a] |
| Control | 1.173±0.037[a] | 1.297±0.027[a] | 1.975±0.097[a] |
| Treated | 1.275±0.070[b] | 1.340±0.028[a] | 2.272±0.142[a] |

*Means followed by different letters in a column indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$

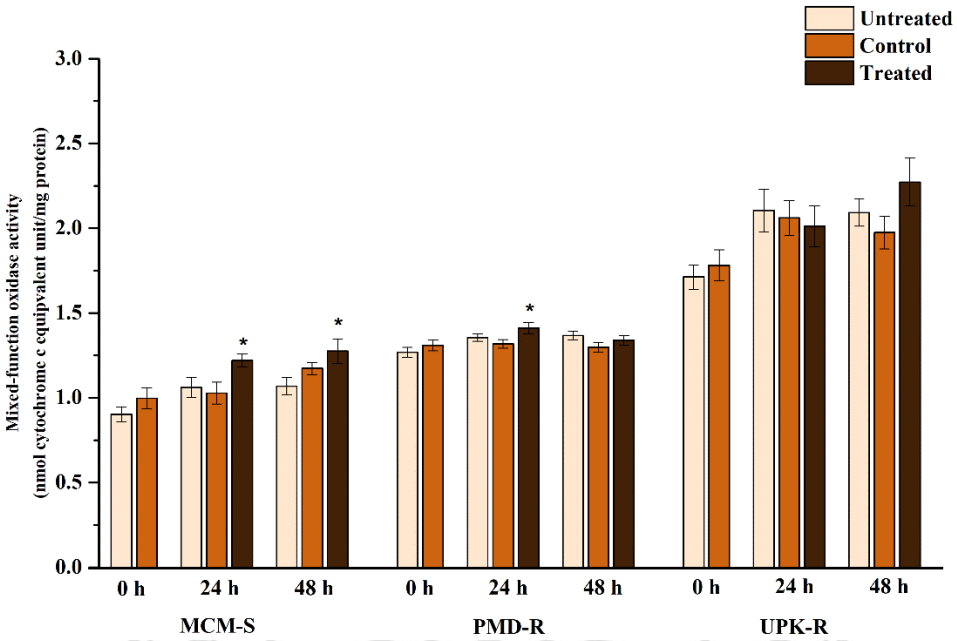


**Figure 3.25** MFO activity in the whole body homogenates of the adults pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains exposed to *P. crispum* oil ($LD_{50}$) after 24 and 48 h. Each bar represents mean±SE of six determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

### 3.3.3.2.3.4 ACP and ALK activity

The activities of ACP in the control and untreated adults were slightly decreased with increasing exposure time (0 to 24 h) in all strains of mosquitoes. After 24 and 48 h of exposure with *P. crispum* oil, the levels of ACP activity in the adult females of MCM-S, PMD-R, and UPK-R were unchanged, when compared to those of the controls (Table 3.20 and Figure 3.26). However, the levels of ALK activity in the treated adults of PMD-R and UPK-R strains (24-h time point), and MCM-S strain (48-h time point) were significantly increased comparing to those of the controls ($P < 0.05$) (Table 3.21 and Figure 3.27).

**Table 3.20** ACP activity in adults of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | ACP activity (Mean±SE, nmol *p*-nitrophenol/min/mg.pro)* | | |
|---|---|---|---|
| | MCM-S | PMD-R | UPK-R |
| 0 hr | | | |
| Untreated | 135.65±4.48[a] | 109.69±2.55[a] | 155.67±1.48[a] |
| Control | 138.34±3.32[a] | 107.57±1.82[a] | 161.46±4.63[a] |
| 24 hr | | | |
| Untreated | 112.59±1.98[a] | 88.33±2.27[a] | 137.21±2.30[a] |
| Control | 117.23±3.92[a] | 86.72±3.42[a] | 140.76±1.84[a] |
| Treated | 108.29±2.81[a] | 88.14±4.77[a] | 141.16±5.10[a] |
| 48 hr | | | |
| Untreated | 91.90±2.93[a] | 86.42±1.66[a] | 133.18±5.55[a] |
| Control | 96.41±3.30[a] | 89.56±3.96[a] | 135.92±4.22[a] |
| Treated | 97.59±1.66[a] | 92.99±3.01[a] | 134.16±1.84[a] |

*Means followed by different lettersin a column indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$

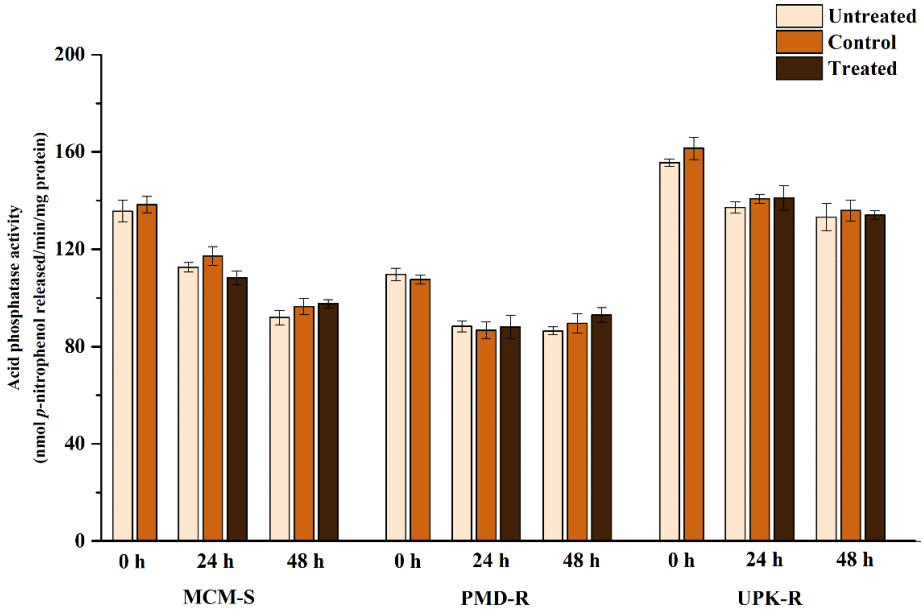


**Figure 3.26** ACP activity in the whole body homogenates of the adults pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains exposed to *P. crispum* oil ($LD_{50}$) after 24 and 48 h. Each bar represents mean±SE of six determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

**Table 3.21** ALK activity in adults of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | ALK activity Mean±SE (nmol *p*-nitrophenol/min/mg.pro)* | | |
|---|---|---|---|
| | MCM-S | PMD-R | UPK-R |
| 0 hr | | | |
| Untreated | 11.37±0.77[a] | 12.14±0.57[a] | 9.46±0.41[a] |
| Control | 12.14±1.26[a] | 13.41±0.88[a] | 9.54±0.52[a] |
| 24 hr | | | |
| Untreated | 11.66±0.56[a] | 11.33±0.55[a,b] | 8.68±0.30[a] |
| Control | 10.83±0.77[a] | 10.08±0.78[a] | 9.11±0.28[a] |
| Treated | 13.56±1.09[a] | 13.30±0.80[b] | 11.37±0.64[b] |
| 48 hr | | | |
| Untreated | 11.07±0.44[a,b] | 12.31±0.57[a] | 10.27±0.12[a] |
| Control | 10.59±0.68[a] | 11.55±0.75[a] | 10.88±0.61[a] |
| Treated | 13.53±1.01[b] | 12.42±0.71[a] | 10.71±0.41[a] |

*Means followed by different letters in a column indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$

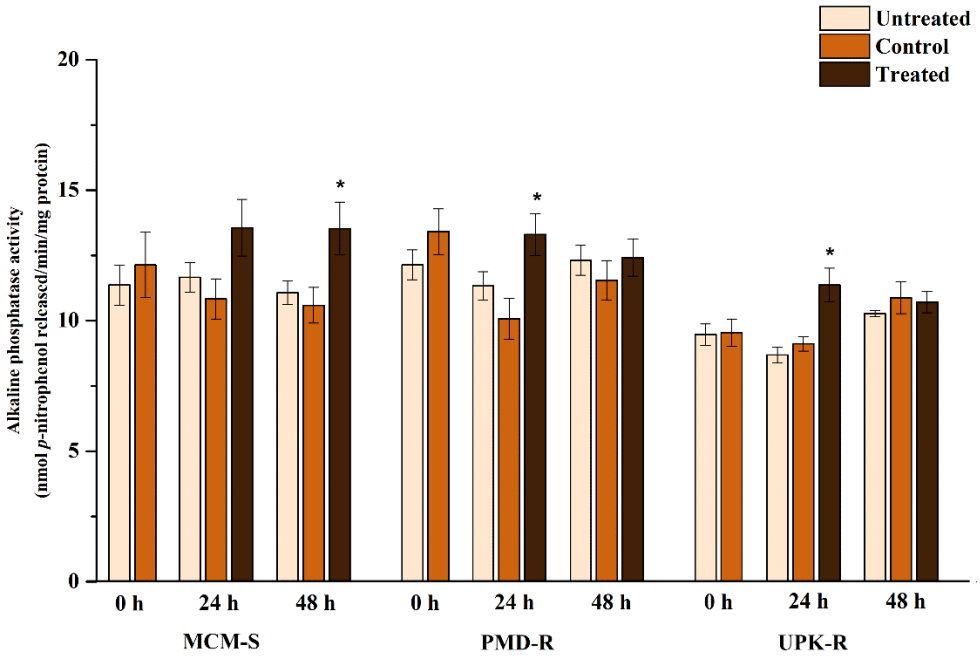


**Figure 3.27** ALK activity in the whole body homogenates of the adults pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains exposed to *P. crispum* oil ($LD_{50}$) after 24 and 48 h. Each bar represents mean±SE of six determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

### 3.3.3.2.3.5 AChE activity

The effects of *P. crispum* oil on AChE activity in MCM-S, PMD-R, and UPK-R strains are shown in Table 3.22 and Figure 3.28. After 24 h of treatment, the level of AChE activity was significantly decreased in only treated PMD-R strain ($P < 0.05$). However, significant decreases of AChE activity were found in all treated adults, MCM-S, PMD-R, and UPK-R, after 48 h of exposure when compared with those of the controls ($P < 0.05$).

**Table 3.22** AChE activity in adults of *Ae. aegypti*, pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains

| Exposure time/Group | AChE activity (Mean±SE, nmol/min/mg.pro)* | | |
|---|---|---|---|
| | MCM-S | PMD-R | UPK-R |
| 0 hr | | | |
| Untreated | 10.71±0.23[a] | 10.07±0.19[a] | 11.80±0.24[a] |
| Control | 10.68±0.14[a] | 9.76±0.21[a] | 11.55±0.32[a] |
| 24 hr | | | |
| Untreated | 9.82±0.21[a] | 7.96±0.36[a] | 9.57±0.27[a] |
| Control | 9.69±0.28[a] | 7.95±0.16[a] | 9.24±0.27[a] |
| Treated | 9.72±0.21[a] | 6.67±0.35[b] | 9.30±0.24[a] |
| 48 hr | | | |
| Untreated | 11.63±0.16[a] | 8.70±0.18[a] | 9.40±0.15[a] |
| Control | 11.45±0.22[a] | 8.42±0.10[a] | 9.15±0.17[a] |
| Treated | 9.86±0.18[b] | 6.87±0.16[b] | 7.74±0.15[b] |

*Means followed by different letters in a column indicate statistically significant difference at 95% confidence level (Tukey's HSD test or independent simple *t*-test; $P < 0.05$

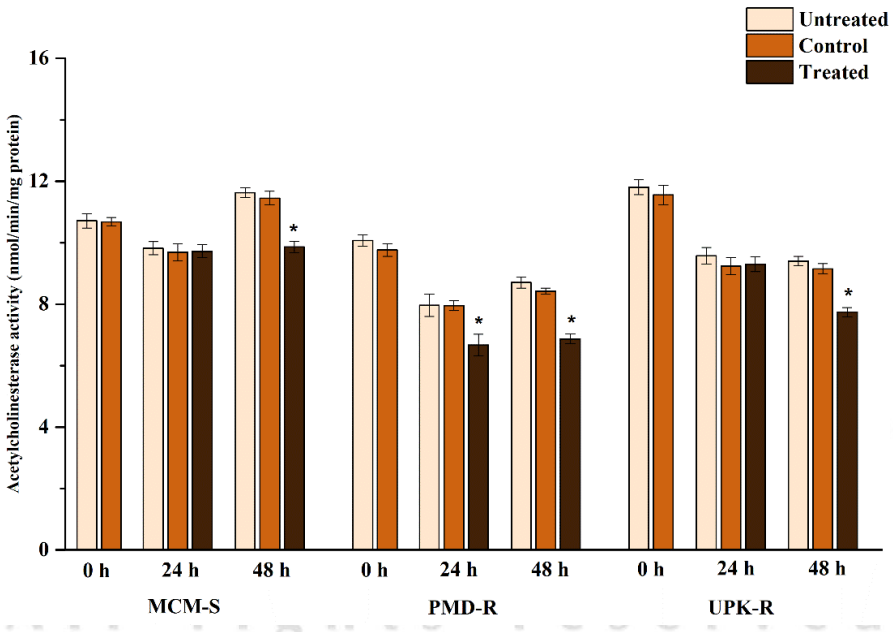


**Figure 3.28** AChE activity in the whole body homogenates of the adults pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains exposed to *P. crispum oil* ($LD_{50}$) after 24 and 48 h. Each bar represents mean±SE of six determinations using samples from different preparations. The star (*) showed significance of mean differences ($P < 0.05$, Tukey's HSD test) between untreated, control, and treated groups.

### 3.4 Chemical composition of *P. crispum* oil

GC-MS analysis of the chemical contents in the most effective product, *P. crispum* fruit oil, revealed the presence of 19 chemicals, accounting for 98.25% of the total oil (Table 3.23 and Figure 3.29). The principal constituents of *P. crispum* oil were thymol (42.41%), *p*-cymene (27.71%), and *γ*-terpinene (20.98%), followed by minor amount of *β*-pinene with 2.54% composition. The remaining 15 compounds ranged from a composition of 0.05-0.97%.

**Table 3.23** Chemical constituents of *P. crispum* fruit oil

| No | RT[a] | Compounds | Area, % | KI[b] |
|---|---|---|---|---|
| 1 | 4.56 | *α*-Thujene | 0.37 | 935 |
| 2 | 4.69 | *α*-Pinene | 0.48 | 944 |
| 3 | 5.34 | *β*-Pinene | 2.54 | 986 |
| 4 | 5.42 | *β*-Myrcene | 0.92 | 991 |
| 5 | 5.76 | 3-Caren | 0.11 | 1014 |
| 6 | 6.05 | *p*-Cymene | 27.71 | 1034 |
| 7 | 6.14 | Sabinene | 0.61 | 1041 |
| 8 | 6.55 | *γ*-Terpinene | 20.98 | 1068 |
| 9 | 6.69 | 4-Thujanol, stereoisomer | 0.09 | 1077 |
| 10 | 6.90 | *α*-Terpinolene | 0.05 | 1090 |
| 11 | 7.15 | 4-Thujanol | 0.08 | 1116 |
| 12 | 8.05 | 4,5-Epoxy-1-isopropyl-4-methyl-1-cyclohexene | 0.08 | 1168 |
| 13 | 8.34 | 4-Terpineol | 0.38 | 1188 |
| 14 | 8.62 | *α*-Terpineol | 0.18 | 1216 |
| 15 | 9.93 | Thymol | 42.41 | 1307 |
| 16 | 10.02 | *p*-Thymol | 0.97 | 1314 |
| 17 | 17.64 | Durenol | 0.10 | 1957 |
| 18 | 18.40 | Carvacrol | 0.09 | 2035 |
| 19 | 18.57 | *p*-Cymen-7-ol | 0.10 | 2053 |
| Total identified | | | 98.25 | |

[a]Retention time (min)

[b]Kovats index relative to *n*-alkane ($C_8$-$C_{40}$) on a DB-5MS column

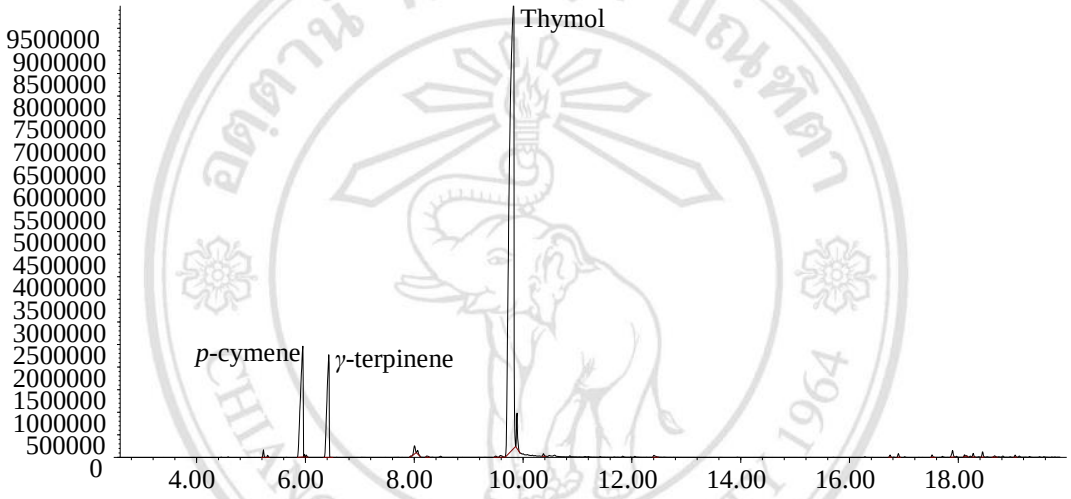


**Figure 3.29** GC/MS total ion chromatograms of *P. crispum* fruit oil.