The Effect of Chemical Composition and Bioactivity of Several Essential Oils on *Tenebrio molitor* (Coleoptera: Tenebrionidae)

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**ABSTRACT.** The major chemical components of four essential oils (EOs) extracted from dry leaves of *Citrus limonum*, *Cymbopogon citratus*, *Litsea cubeba*, and *Muristica fragrans* were analyzed with gas chromatograph-mass spectrometer and their fumigant, contact, and repellent activities against 10th instar and adults of *Tenebrio molitor* were also assayed. The results indicated that the major constituents of *C. limonum* and *C. citratus* were D-limonene (38.22%) and 3,7-dimethyl-6-octenal (26.21%), while which of *L. cubeba* and *M. fragrans* were (E)-3, 7-dimethyl-2, 6-octadienal (49.78%) and (E)-cinnamaldehyde (79.31%), respectively. Contact activities of *L. cubeba* and *C. limonum* with LC₅₀ values of 21.2 and 13.9 μg/cm² at 48 h and repellence activities (>89.0% repellence indexes) (P < 0.05) at 12 h on 10th instar were better than those of the other two EOs. Nevertheless, the fumigation activities of *L. cubeba* on 10th instar and adults (LC₅₀ = 2.7, 3.7 μl/liter) were stronger than those of *C. limonum* (LC₅₀ = 10.9, 12.0 μl/liter) at 96 h and significant (not overlapping confidence intervals). The EOs of *L. cubeba* and *C. limonum* have clearly elongated the growth and development of larvae, egg, and slightly shorten pupae and adults of *T. molitor* compared with the control. The main active ingredients of *L. cubeba* and *C. limonum*, including D-limonene and β-pinene, were demonstrated to inhibit the activities of AChE and enhance the toxicities on 10th instar of *T. molitor*. These results indicate that the EOs of *L. cubeba* and *C. limonum* could have great potential as botanical insecticides against *T. molitor*.

**Key Words:** *Tenebrio molitor*, fumigant toxicity, growth, development, AChE activity

*Tenebrio molitor* L. (Coleoptera: Tenebrionidae) is one of the mainly stored product pests of *Juncus effuses* L. (Poales: Juncaceae), which is being widely cultivated in southwest China and is acted as an important straw mat for summer sleeping, such as tatamis (Li et al. 2009, Wang et al. 2014). Some means, including hot treatment, sun treatment, and fumigation with some chemicals, have been attempted to control them. However, many negative consequences (pest resistance, residual toxicities, environmental pollution, and so on) have limited the application of chemical control (Wang et al. 2010). Plant-derived natural chemicals, known as secondary metabolites, are effective in their roles as possible alternatives to synthetic chemical insecticides, and many of them have displayed numerous pesticidal biological activities (Ngassoum et al. 2007, Rossi et al. 2009, Nesci et al. 2011). Recently, the plant oils characterized by a strong volatile and lower density than water, which emer...
Clevenger apparatus (Beijing Shi Ji Hui Yuan Technology Co., LTD) for 3–4 h, dried over anhydrous sodium sulfate and refrigerated at 4°C (Mahnaz et al. 2012).

The oils were analyzed by gas chromatography-mass spectrometry (GC-MS) with the some modification methods described by Wang et al. (2014). The GC oven temperature was kept at 50°C for 2 min, programmed at 5°C min⁻¹ ramps to 240°C and then held about 10 min. The temperature of injector and detector was 250°C. He was carrier gas (1 ml min⁻¹, split ratio 1:50) and the samples and n-alcohols (consecutive C8-C40, bought from Accustandard, Inc. www.accustandard.com) were diluted in acetone (injection of 2 μl). Mass spectra were recorded at 70 eV, and the mass range was m/z 30-600 amu. The compounds were identified by comparing the retention indices (Kovats indices) with their mass spectra stored in the MS database (NIST98 MS DATA) (Haouas et al. 2012) and the relative percentage amounts were according to the GC peak areas.

Contact Activity. The concentration of 60 μg/cm² for each EO diluted by acetone on the Whatman No. 1 filter papers (9 cm in diameter) were prepared and 1 ml acetone was acted as the blank control. After evaporating solvent about 10 min at 25°C, filter paper filled with EOs or not was then placed inside a glass Petri dish with 30 10th-instar T. molitor, coating Teflon on inner wall of case in escaping. Quickly covered Petri dish and cultivated in an incubator at 27 ± 2°C and 60 ± 5% RH and in darkness. Mortality and corrected mortality were calculated at 24 and 48 h after treatment.Contact toxicities of L. cubeba and C. limonum EOs were assayed with a series of concentrations of 5, 10, 20, 40, 80, 160, and 0 (control) μg/cm² with the same method (Zhao et al. 2012, Wang et al. 2014). Each treatment was triplications and the LC50 and LC95 (lethal concentration) values, and their 95% confidence intervals (CIs) at 24 and 48 h after treatments were calculated with POLO2.0 (Leora Software, www.leorasoftware.com), respectively.

Repellent Activity. The experimental method was described by Wang et al. (2006, 2014) with some modifications. Whatman no. 1 filter papers (diameter 12.5 cm) were cut in half and each EO (including 300, diluted by acetone on the Whatman No. 1 filter paper strips (1.5 cm by 6 cm) and holed with a line adhesive at the center of each filter paper disc. The dishes were then covered and transferred into an incubator. Triplications were held for each concentration. After 12, 24, and 48 h, the number of larvae present at each amount of treated or control halves was counted. The distribution coefficient was calculated with the following formula:

\[ DC = \frac{C - T}{C + T} \times 100\% \]

The C value is behalf of the number of larvae on control half and T is the number of larvae on treated half. Positive values stood for repellency and negative values expressed attractancy.

Fumigant Activity. The fumigant activities of EOs on 10th instar, and adults of T. molitor were conducted with sealing jar (Deng et al. 2004, Wang et al. 2014). Whatman No.1 filter papers were made into filter paper strips (1.5 cm by 6 cm) and holed with a line adhesive at the sealing of plastic film to avoid contact with the 500 ml vial bottom. Thirty 10th-instar larvae were transferred into the jar, followed by adding 10 μl tested EOs in filter paper strips (concentration of 20 μl/liter) and quickly covered the vial with sealing of plastic film. Triplications were set, and treated vials were kept at 27 ± 2°C and 60 ± 5% RH. Mortalities and adjusted mortalities after 48 and 96 h were calculated by using Abbott’s (1925) formula. The toxicities of the two stronger activities EOs on larvae and adults were assayed with the series of concentrations of 2, 4, 8, 16, 32, and 0 (control) μl/liter using the same method over mentioned. The toxicities of EOs of LC50, LC95 values, and their 95% CI at 48 and 96 h were calculated as the description of contact activity.

Growth and Development. Thirty sixth instar were transferred into a plastic case with length by width by height (250 mm by 100 mm by 50 mm), and the two stronger active EOs, including L. cubeba and C. limonum, were added in feed with the concentration of 2 μl/liter until 13th-instar larvae. The growth and development of EOs on larva, pupae, adult, and egg of T. molitor were counted. Three replications were set for each treatment.

Fumigant Toxicity of Mainly Active Ingredients. The fumigant toxicity of mainly active ingredient, including D-limonene and β-pinene of C. limonum and L. cubeba, which were all purchased from Aladdin reagent (Shanghai) co. Ltd. (http://www.aladdin-e.com/), were assayed on 10th-instar T. molitor with method 2.5. The concentrations of all tested single or mix of compounds (ν/ν = 1:1) were set for 2, 4, 8, 16, 32, and 0 (control) μl/liter. The toxicities of EOs of LC50, LC95 values, and their 95% CI after 48–96 h were calculated as the description of contact activity.

AChE Activity Assay. When 15 10th-instar larvae were fumigated with the mixture of D-limonene plus β-pinene, sole D-limonene, or β-pinene (with the concentrations of LC50 at 48 h, respectively) at 12, 24, 36, 48, 60, 72, and 96 h after treatment, the tested insect were frozen with liquid nitrogen and homogenized in 10 ml of 0.1 M ice-cold phosphate buffer (pH 7.0) using a mortar. Homogenates were centrifuged at 7,000 rpm for 15 min at 0°C, and the supernatants were used for the enzyme source and acetylcholine bromide as substrate. Enzyme aliquots (50 μl) and 100 μl 5,5'-Dithiobis-(2-nitrobenzoic acid), DTNB (0.01) were added to 0.1 M phosphate buffer (pH 8.0, 2.8 ml) and incubated at 37°C about 15 min. Acetylcholine bromide (30 μl) was added into the system to react at 37°C for 10 min. The inhibitions of AChE activities treated with compounds were displayed with changes of absorbance at 412 nm with UV 2000-Spectrophotometer (Unic [Shang Hai] Instruments Incorporated), and all the experiments were set for triplicate (Yeeom et al. 2012, Wang et al. 2014). Inhibition percentage of AChE activity was obtained with the following formula:

\[ \text{AChE inhibition} (%) = \frac{OD_B - OD_T}{OD_B} \times 100\% \]

where OD is the optical density of blank enzyme and OD is the optical density of treatment.

Statistical Analyses. In this article, the fumigant, contact, repellent activities on test insects, growths, and developments of larvae, pupae, adult, and egg of T. molitor were compared using analysis of variance (Duncan’s test for multiple – comparison, P < 0.05) with SPSS v.17.0 software package (IBM, www.ibm.com) in Microsoft Windows 7 operating system (www.microsoft.com). And the figure of growth and development of larvae, pupae, adult, egg, and total of T. molitor was drawn by SigmaPlot v.10.0 software (www.sigmaplot.com).

Results

The Main Ingredients of Test EOs. Based on the GC-MS data (Table 1), the main ingredients of C. limonum contained D-limonene (38.22%) and β-pinene (19.74%), and C. citratus were mainly constituted with 3,7-dimethyl-6-octanal (26.21%) and 2,6-octadien-1-ol, 3,7-dimethyl (20.42%). L. cubeba mainly contained D-limonene (20.22%) and (E)-7,7-dimethyl-2, 6-octadienal (49.78%), and M. fragrans composed of methyl salicylate (6.79%) and (E)-cinnamaldehyde (79.31%).

Contact Activity. Good contact activities of EOs on 10th-instar T. molitor are listed in Table 2. The activity of L. cubeba was the highest, followed by C. limonum. Nevertheless, the contact activities of the other two EOs were not perfect with occurring <50% adjusted mortalities during the experimental period.

The toxicities of EOs of L. cubeba on 10th instar of T. molitor was recorded with LC50 value (19.6 μg/cm²) at 24 h after treatment and was not equitoxic with that of C. limonum (42.2 μg/cm²) (not overlapping...
were significant stronger than those of C. limonum, Cy. citratus, L. cubeba, and M. fragrans, whereas the toxicity of C. limonum (21.2 µg/cm²) was quickly promoted and equitoxic with that of L. cubeba (13.9 µg/cm²) posttreatment 48 h (overlapping CIs) (Table 3). No test insect mortality was observed in the control.

**Repellent Activity.** The repellent activities of L. cubeba and C. limonum were stronger than those of the other two EOs, which were all almost <50% repellence indexes on 10th instar of T. molitor. The effects of all treatments were weaker and weaker with lower concentration and elongation of experiment (Table 4).

**Fumigant Activity.** The fumigant activity of L. cubeba on the 10th instar of T. molitor was the strongest from 48 to 96 h after treatment, followed by C. limonum, M. fragrans, and Cy. citratus. No test insect mortality was observed in the control.

**Growth and Development.** The results indicated that the growth and development of larvae of T. molitor treated with EOs were clearly elongated with 52.9 and 52.3 d for L. cubeba and C. limonum, respectively, and significant with that of control (39.6 d, P < 0.05). On the contrary, the growth and development of pupae of T. molitor treated with EOs (with 6.2 d for L. cubeba and 6.3 d for C. limonum) were shorter than that of control with 7.4 d (P < 0.05). A generation of T. molitor treated with L. cubeba (6.3 d) were longer than those of the control (5.2 d) but not significant with C. limonum (6.0 d) (P > 0.05). A generation of growths and developments treated with EOs (105.3 d for L. cubeba and 106.2 d for C. limonum, respectively) were significant with that of control 95.7 d (P < 0.05) (Fig. 1).

### Table 1. Chemical constituents and yields (in %) of EOs extracted from C. limonum, Cy. citratus, L. cubeba, and M. fragrans

| EO         | Compound                                      | Content (%) | RT (min) | Retention index |
|------------|-----------------------------------------------|-------------|----------|-----------------|
| C. limonum | z-phellandrene                                | 1.54        | 4.59     | 1,001.4         |
|            | z-pine ne                                     | 4.47        | 4.74     | 1,003.7         |
|            | β-pinene                                      | 19.74       | 5.75     | 1,019.5         |
|            | D-limonene                                     | 38.22       | 6.56     | 1,032.1         |
|            | 1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene | 13.08       | 7.18     | 1,041.8         |
|            | (Z)-3,7-dimethyl-2, 6-octadienal              | 2.6         | 10.45    | 1,092.9         |
|            | (E)- 3,7-dimethyl-2, 6-octadienal             | 4.04        | 10.99    | 1,201.2         |
|            | Other compounds                               | 16.31       | —        | —               |
| Cy. citratus| D-limonene                                     | 5.18        | 13.28    | 1,235.0         |
|            | 3,7-dimethyl-6-octenal                        | 26.21       | 17.7     | 1,400.2         |
|            | (R)-3,7-dimethyl-6-octen-1-ol                 | 15.34       | 19.96    | 1,437.4         |
|            | 2,6-octadien-1-ol, 3,7-dimethyl              | 20.42       | 20.94    | 1,453.5         |
|            | (E)-2,6-octadien-1-ol, 3,7-dimethyl-acetate   | 5.01        | 24.05    | 1,605.1         |
|            | (1S-cis)-2,3,5,6,8α-hexahydro-4,7-dimethyl-1-(1-methyle thyl)-naptha line | 4.28 | 27.6 | 1,670.3 |
|            | 4-ethenyl-5,3,4-trimethyl-3-(1-methylethylenyl)-[1R,(1a,3,3a)]-cyclohexanemethanol | 5.94 | 28.49 | 1,686.6 |
|            | Other compounds                               | 17.62       | —        | —               |
| L. cubeba  | 1R-α-pinene                                   | 3.04        | 6.5      | 1,031.2         |
|            | 4-methylene-1-(1-methylethyl)-bicyclo[3,1,0]hexane | 4.11 | 7.85 | 1,052.3 |
|            | 6-methyl-5-hepten-2-one                       | 2.88        | 8.4      | 1,060.9         |
|            | D-limonene                                     | 20.22       | 10.21    | 1,089.1         |
|            | (Z)-3,7-dimethyl-2,6-octadienal              | 10.57       | 19.79    | 1,436.4         |
|            | (E)-3,7-dimethyl-2,6-octadienal              | 49.78       | 21.88    | 1,468.9         |
|            | Caryophyllene                                 | 3.37        | 26.7     | 1,653.8         |
|            | Other compounds                               | 6.03        | —        | —               |
| M. fragrans| benzaldehyde                                  | 0.70        | 5.76     | 1,019.6         |
|            | methyl salicylate                             | 6.79        | 11.95    | 1,215.4         |
|            | 3-phenyl-2-propenal                           | 0.18        | 12.56    | 1,224.4         |
|            | (E)-cinnamaldehyde                            | 79.31       | 14.35    | 1,293.1         |
|            | 3-allyl-6-methoxyphenol                       | 3.93        | 16.45    | 1,281.8         |
|            | 3-phenyl-2-propenoic acid                     | 1.08        | 18.68    | 1,416.3         |
|            | 7-methyl-1-naphthal                           | 0.37        | 21.36    | 1,460.4         |
|            | Other compounds                               | 7.64        | —        | —               |

RT, retention time.

### Table 2. The contact activity of EOs on 10th-instar T. molitor

| EO         | Number of insects tested | Adjusted mortality of T. molitor (%) (± SE) |
|------------|--------------------------|-------------------------------------------|
|            | 24 h                     | 48 h                                      |
| C. limonum | 90                       | 46.7 ± 3.9 b*                             | 65.6 ± 4.0 b*                             |
| Cy. citratus| 90                       | 22.4 ± 2.9 c                             | 46.7 ± 3.9 c                             |
| L. cubeba  | 90                       | 66.7 ± 1.9 a                             | 88.9 ± 2.9 a                             |
| M. fragrans| 90                       | 32.2 ± 2.2 c                             | 47.8 ± 2.8 c                             |
| Control    | 90                       | 0 d                                       | 0 d                                       |
| F6,F10     | —                        | <0.0001                                  | <0.0001                                  |

*Means within a column followed by the different letter are significant (P < 0.05) as determined by Duncan’s test.

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Table 3. The contact toxicity of EOs on 10th instar of *T. molitor*

| EO            | Treatment time (h) | Number of insects tested | LC$_{50}$ (µg/cm$^2$) 95% CI$^a$ | LC$_{95}$ (µg/cm$^2$) 95% CI$^a$ | Slope ± SE | Chi-square (df) | $P^*$ |
|---------------|--------------------|--------------------------|-----------------------------------|-----------------------------------|-----------|----------------|-------|
| *L. cubeba*   | 24                 | 450                      | 19.6 (16.4–23.2)                  | 196.8 (141.4–305.7)              | 1.64 ± 0.13 | 3.48 (4)       | < 0.05 |
|               | 48                 | 450                      | 13.9 (8.8–19.7)                   | 196.2 (105.0–623.1)              | 1.43 ± 0.13 | 6.67 (4)       | < 0.05 |
| *C. limonum*  | 24                 | 450                      | 42.2 (34.9–51.7)                  | 583.2 (368.8–1100.0)             | 1.44 ± 0.13 | 0.84 (4)       | < 0.01 |
|               | 48                 | 450                      | 21.2 (17.4–25.5)                  | 285.5 (192.9–488.3)              | 1.46 ± 0.12 | 1.20 (4)       | < 0.01 |
| Control       |                    | 90                       | —                                 | —                                 | —         | —              | —     |

$^a$ LC$_{50}$ or LC$_{95}$ values are considered significantly different when the 95% CI do not overlap.

*Goodness-of-fit test is significant at $P < 0.05$.

Table 4. Repellent activity of EOs on 10th-instar *T. molitor*

| EO            | Treatment time | Number of insects tested | Repellence index after 12 h treatment (%) (± SE) | Repellence index after 24 h treatment (%) (± SE) | Repellence index after 48 h treatment (%) (± SE) |
|---------------|---------------|--------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| *C. limonum*  | 300           | 600 900                  | 71.9 ± 0.1 b$^a$ 77.7 ± 4.0 b 89.9 ± 1.9 a | 62.2 ± 3.0 b 68.3 ± 4.2 b 83.3 ± 1.9 a | 53.7 ± 2.2 a 63.9 ± 3.6 b 75.0 ± 2.9 a |
| *Cy. citratus*| 300           | 600 900                  | 41.8 ± 2.9 c 51.4 ± 2.9 c 63.1 ± 3.9 b | 53.1 ± 1.6 c 44.5 ± 2.2 c 55.0 ± 3.5 b | 28.4 ± 4.5 b 37.1 ± 2.0 c 45.7 ± 2.8 b |
| *L. cubeba*   | 300           | 600 900                  | 83.1 ± 1.8 a 90.0 ± 1.9 a 95.1 ± 1.0 a | 73.5 ± 2.1 a 80.6 ± 0.6 a 88.5 ± 1.0 a | 24.3 ± 3.0 b 33.7 ± 2.3 c 41.3 ± 1.3 b |
| *M. fragrans* | 300           | 600 900                  | 37.7 ± 1.2 a 51.7 ± 2.5 a 59.1 ± 3.0 b | 41.6 ± 1.0 c 47.1 ± 2.8 c 47.6 ± 2.8 c | 32.4 ± 3.0 c 33.7 ± 2.3 a 41.8 ± 1.3 b |
| Control       |               |                          | 0 d                                   | 0 d                                   | 0 d                                   |
| $F_{4,10}$    | <0.0001       |                          | 110.9                                 | 173.5                                 | 252.0                                 |
| $P$           | <0.0001       |                          | 0.05                                 | 0.05                                 | 0.05                                 |

$^a$ µg/cm$^2$ filter paper.

$^b$ Means within a column followed by the different letter differ significantly ($P < 0.05$) according to analysis of variance (ANOVA). The number of insects tested for each treatment was 90.

Table 5. Fumigant activity of EOs on 10th instar of *T. molitor*

| EO            | Number of insects tested | Adjusted mortality of *T. molitor* (%) (± SE) |
|---------------|--------------------------|-----------------------------------------------|
| *C. limonum*  | 90                       | 55.6 ± 2.2 b$^a$ 73.3 ± 1.9 b$^a$ |
| *Cy. citratus*| 90                       | 30.0 ± 1.9 c 53.3 ± 1.8 c |
| *L. cubeba*   | 90                       | 75.6 ± 2.9 a 92.2 ± 1.1 a |
| *M. fragrans* | 90                       | 32.2 ± 2.2 c 58.9 ± 2.9 c |
| Control       | 90                       | 0 d                                             |
| $F_{4,10}$    | <0.0001                  | 183.3                                           |
| $P$           | <0.0001                  | < 0.0001                                         |

$^a$ Means within a column followed by the different letter are significant ($P < 0.05$) as determined by Duncan’s test.

Table 6. The fumigant toxicity of EOs on 10th instar of *T. molitor*

| EO            | Treatment time (h) | Number of insects tested | LC$_{50}$ (µl/liter) 95% CI$^a$ | LC$_{95}$ (µl/liter) 95% CI$^a$ | Slope ± SE | Chi-square (df) | $P^*$ |
|---------------|--------------------|--------------------------|---------------------------------|---------------------------------|-----------|----------------|-------|
| *L. cubeba*   | 48                 | 450                      | 4.5 (3.7–5.3)                   | 50.0 (35.0–80.6)                | 1.57 ± 0.13 | 1.94 (4)       | < 0.05 |
|               | 96                 | 450                      | 2.7 (2.3–3.2)                   | 22.2 (16.7–32.3)                | 1.80 ± 0.15 | 3.05 (4)       | < 0.05 |
| *C. limonum*  | 48                 | 450                      | 29.2 (21.1–46.5)                | 781.3 (325.3–1104.0)            | 1.15 ± 0.14 | 0.51 (4)       | < 0.01 |
|               | 96                 | 450                      | 10.9 (8.8–13.9)                 | 209.2 (117.6–479.3)             | 1.28 ± 0.12 | 0.84 (4)       | < 0.05 |
| Control       |                    | 90                       | —                               | —                               | —         | —              | —     |

$^a$ LC$_{50}$ or LC$_{95}$ values are considered significantly different when the 95% CI do not overlap.

*Goodness-of-fit test is significant at $P < 0.05$.

Table 7. The fumigant toxicity of EOs on adults of *T. molitor*

| EO            | Treatment time (h) | Number of insects tested | LC$_{50}$ (µl/liter) 95% CI$^a$ | LC$_{95}$ (µl/liter) 95% CI$^a$ | Slope ± SE | Chi-square (df) | $P^*$ |
|---------------|--------------------|--------------------------|---------------------------------|---------------------------------|-----------|----------------|-------|
| *L. cubeba*   | 48                 | 450                      | 5.3 (4.4–6.3)                   | 59.3 (41.1–97.1)                | 1.57 ± 0.13 | 1.44 (4)       | < 0.05 |
|               | 96                 | 450                      | 3.7 (3.0–4.5)                   | 47.6 (32.8–78.9)                | 1.48 ± 0.13 | 1.70 (4)       | < 0.05 |
| *C. limonum*  | 48                 | 540                      | 25.3 (18.7–38.5)                | 636.0 (280.1–2263.8)            | 1.17 ± 0.13 | 0.41 (4)       | < 0.01 |
|               | 96                 | 450                      | 12.0 (9.5–15.9)                 | 289.0 (149.7–764.0)             | 1.19 ± 0.12 | 1.21 (4)       | < 0.05 |
| Control       |                    | 90                       | —                               | —                               | —         | —              | —     |

$^a$ LC$_{50}$ or LC$_{95}$ values are considered significantly different when the 95% CI do not overlap.

*Goodness-of-fit test is significant at $P < 0.05$. 
**Fumigant Toxicities of Mainly Active Ingredients.** The toxicity of the mixture of D-Limonene plus β-pinene on 10th instar of *T. molitor* was the strongest at 48 h after treatment and significant with the other two sole compounds (not overlapping CIs), followed by the D-Limonene and significant with β-pinene (not overlapping CIs). The toxicities of D-Limonene on 10th instar of *T. molitor* were quickly enhanced and seemed to be equitoxic with that of mix of D-Limonene plus β-pinene (2.0 µl/liter) 96-h posttreatment (overlapping CIs) (Table 8). No test insect mortality was found in the control.

**Inhibitions of Mainly Active Ingredient of EOs on AChE Activity.** The mix of D-limonene plus β-pinene displayed the strongest inhibition on AChE activity in 10th instar of *T. molitor* and was very significant with other treatments from 12 h to 96 h (*P* < 0.01), which of AChE-inhibition rate was <50% before 48 h; however, the effects of D-limonene on AChE activities were clearly improved and significant with those of β-pinene from 60 to 96 h (*P* < 0.01) (Fig. 2).

**Discussion**

EO is considered to be an alternative means of controlling many pests (Wang et al. 2010). There were some early reports on the insecticidal activities of EOs from *C. limonum* (Ponce et al. 2004, Moreira et al. 2005) and *L. cubeba* (Jiang et al. 2009, Seo et al. 2009) on some storage insects. The results of this study showed that tested EOs had strong insecticidal activities on adult and/or nymphal stages of *T. molitor*. The EOs of *L. cubeba* and *C. limonum* have also displayed strong insecticidal toxicities on larvae than adults of *T. molitor*, which were consistent with some early researches (Pavela 2008, Jiang et al. 2009, Seo et al. 2009). Our data also demonstrated that EOs of *L. cubeba* and *C. limonum* possessed strong contact and repellent activities on *T. molitor*.

There were some previous researches reported that effects of plant EOs are related to their chemical ingredients (Ngassoum et al. 2007, Ko et al. 2009), including pulegone, linalool, eugenol, thymol, cymol, methyl chavicol, and so on, which were known to be poisonous to many insects (Park et al. 2006b, Thongdon and Inprakhon, 2009). The ingredients of tested EOs in this study were included in following chemical compositions: α-pinene, β-pinene, D-limonene, (E)-3,7-dimethyl-, 2, 6-octadienal, and so on, which have been reported to be toxic to some insects (Park et al. 2006a, Zapata and Smagghe 2010). We also found an interesting phenomenon that the main ingredient,
D-limonene from *C. limonum* collected during the 2012 autumn from Wenjiang district reached 64.53% (Wang et al. 2014). However, the content of limonene from *C. limonum* collected in the Lushan County, Ya’an City, in this article was only 38.22%, which could be explained that the ingredients of same plant at different places and seasons could metabolism different compounds, yields of EOs and displayed distinct activities (Hussain et al. 2010). We found that the growth and development of larvae of *T. molitor* were elongated and pupae were slightly shortened compared with those of the control. The phenomenon was partly consistent with the early results. Qin et al. (2010) reported that the EO from the leaves of *Piper sarmentosum* could markedly prolong the developmental duration of *Brontispa longissima* in different instars, which of the control was 25.7d, whereas the *P. sarmentosum* EOs treatment from 100 mg/liter to 2,000 mg/liter were elongated from 27.69 d to 40.26 d. Meanwhile, a generation of the control was only 43.34 d, but which of the tested EOs were prolonged from 48.06 d to 73.58 d, respectively.

Another stirring result that the mix of D-limonene and β-pinene have been synergy to improve the toxicities and AChE-inhibition on 10th instar of *T. molitor* compared with single D-limonene and β-pinene have been demonstrated (Table 8 and Fig. 2). The results were supported with some previous reports (Feng et al. 1995, Maurya et al. 2012). So the effects of multi-ingredients of EOs could interact and enhance the ability of inhibitions on the targets, such as AChE, mixed-functional oxidase, carboxylesterase, and glutathione S-transferase, and so on and result in the better efficacies on the pests.

In general, the EOs extracted from *C. limonum* and *L. cubeba* have potent fumigant, content, and repellent activities and might be used for effective managements of *T. molitor* occurred in *J. effuses*. Our conclusions were necessarily tenuous, and further studies were required since the information about the safe of EOs resided in *J. effuses* were not clearly even though with heat-treatment. However, the above results have provided a foundation for subsequent efforts for exploiting the safe, environment friendly agency to effectively manage the insect.

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