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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 *Cy. citrates* 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 LC_{50} values of 21.2 and 13.9 μg/cm^2 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 ($LC_{50} = 2.7$, 3.7 μl/liter) were stronger than those of *C. limonum* ($LC_{50} = 10.9$, 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 mainly active ingredients of *L. cubeba* and *C. limonum*, including D-limonene and β-pinene, were demonstrated to coinhibit the actives 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 embraced some insecticidal activities, have become a hotspot in pesticide research as possible alternatives to synthetic chemical insecticides (Copping and Menn 2000, Stefanazzi et al. 2011). There are some early reports that essential oils (EOs) have the potential as insecticide to control some insects or mites. Oyedele et al. (2002) reported that the ointment and cream formulations of lemongrass oil displayed the good repellency active on *Ae. aegypti* L., and 1% solution (v/v) and 15% cream (v/w) and ointment preparations of the oil exhibited ≥50% repellency lasting 2–3 h. Liu et al. (2007) also reported the EOs extracted from 30 Chinese medicinal herbs, including *Artemisia argyi*, *Dictamnus dasycarpus*, *Evodia rutaecarpa, Litsea cubeba, Narcissus tazetta* var. *chinensis,* and so on*,* have exhibited insecticidal or feeding-deterrent activities against two stored-grain insects *Sitophilus zeamais* and *Tribolium castaneum.* Williamson et al. (2007) asserted that lemon oil showed good insecticidal active on house dust mites as control agency. Hanifah et al. (2011) researched the acaricidal activity of *Cymbopogon citratus* EO on house dust mites and found that it was stronger (over 91% topical and contact mortalities with 50% diluted EO) than that of neem (only 40.3% topical mortalities and 15.7% contact mortalities) on the *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* dealt with the same concentration and exposure time. Phasomkusolsil and Soonwera (2011) alleged that the EO of *Cy. citratus* showed the repellency against *An. dirus* with ED_{50} at <0.068 mg/cm^2. Furthermore, the *Cy. citratus* gave strong effective dose (ED_{50}) values at <0.003 mg/cm^2 when tested against *Cx. quinquefasciatus*. Yang et al. (2014) also declared that the EO of *L. cubeba* possessed strong contact toxicity against the cigarette beetle *Lasioderma serricorne* adults and the booklouse *Liposcelis bostrychophila*, with LD_{50} values of 27.33 μg per adult and 71.56 μg/cm^2, respectively, and also showed strong fumigant toxicity against the two stored product insects with LC_{50} values of 22.97 and 0.73 mg/liter, respectively.

Therefore, the objective of this study was to detect on the main ingredients of EOs extracted from *Citrus limonum* (Rutales: Rutaceae)*, Cy. citratus* (Ranales: Lauraceae), *L. cubeba* (Ranales: Lauraceae), and *Muristica fragrans* (Magnoliales: Myristicaceae) and assayed the fumigant, contact, repellent activities, and growth and development, and the effects on AChE activities on adults and 10th instar of *T. molitor* to ensure the potential of the tested EOs as effective alternative insecticides of synthetic insecticides against the beetle occurring storage.

Materials and Methods

Insect. *T. molitor* was obtained from laboratory cultures and maintained in darkness in incubators at 27 ± 2°C and 60 ± 5% relative humidity (RH). Larvae and adults were all reared with a mix of wheat bran, maize powder, and peanut cake at a 7:0.5:1 weight proportion with 15% water. Successively rose three generations, and the 10th instar and adult eclosion 2 d were employed in all experiments.

EO Extraction and Mainly Ingredient Analysis. Our team collected the leaves of *C. limonum, Cy. citratus, L. cubeba,* and *M. fragrans* from a Chinese herbal medicine planting bases of Lushan County, Ya'an city, China, located at 30° 03′ 42″N, 103° 01′35″ E during the summer season of 2013 and extracted the EOs from leaves using a modified

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-alkanes (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/cm^2 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 in case of 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/cm^2 with the same method (Zhao et al. 2012, Wang et al. 2014). Each treatment was triplications and the LC_{50} and LC_{95} (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 as 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, 600, and 900 µg/cm^2, prepared by dissolving different volumes EO into 1 ml acetone) or only acetone (as the control) was applied to half a filter-paper disc for each treatment. The treatments were air dried at about 26°C for 12 min to evaporate the solvent completely and then pasted the treated and untreated halves together on the opposites. Finally, put it into Petri dishes' bottom (diameter 12.5 cm) and coated Teflon on inner wall to stop escaping. Thirty 10th instars were released separately 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.

$$\mathrm{DC} = \frac{C - T}{C + T} \times 100\%$$

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 LC_{50}, LC_{95} 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 repetitions 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 (v/v = 1:1) were set for 2, 4, 8, 16, 32, and 0 (control) µl/liter. The toxicities of EOs of LC_{50}, LC_{95} 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 LC_{50} 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 (Yeom et al. 2012, Wang et al. 2014). Inhibition percentage of AChE activity was obtained with the following formula:

$$\text{AChE inhibition}\,(\%) = \frac{\mathrm{OD_B} - \mathrm{OD_T}}{\mathrm{OD_B}} \times 100$$

where OD_B is the optical density of blank enzyme and OD_T 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. citrates* were mainly constituted with 3,7-dimethyl-6-octenal (26.21%) and 2,6-octadien-1-ol, 3,7-dimethyl (20.42%). *L. cubeba* mainly contained D-limonene (20.22%) and (E)-3, 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 toxicity of EOs of *L. cubeba* on 10th instar of *T. molitor* was recorded with LC_{50} value (19.6 µg/cm^2) at 24 h after treatment and was not equitoxic with that of *C. limonum* (42.2 µg/cm^2) (not overlapping

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	α-phellandrene	1.54	4.59	1,001.4
	α-pinene	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)-naphthalene	4.28	27.6	1,670.3
	4-ethenyl-α,α,4- trimethyl-3- (1-methylethenyl)- [1R-(11α,3α,4β)]-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	1,434.6
	(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-naphthol	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[a]	65.6 ± 4.0 b[a]
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
$F_{4,10}$	—	96.5	110.9
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.

CIs), whereas the toxicity of *C. limonum* (21.2 μg/cm^2) was quickly promoted and equitoxic with that of *L. cubeba* (13.9 μg/cm^2) 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 (Table 5).

The toxicities of *L. cubeba* on 10th instar and adults of *T. molitor* were significant stronger than those of *C. limonum* (not overlapping CIs). Meanwhile, the toxicities of *L. cubeba* on 10th instar (2.7 μl/liter) and adults (3.7 μl/liter) were almost equitoxic (overlapping CIs) at 96 h after treatment, respectively (Tables 6 and 7). 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 ($P < 0.05$). The developmental stages of adults dealt with EOs or not were not different, which of the control, *L. cubeba,* and *C. limonum* were 43.5, 39.8, and 41.6 d, respectively ($P > 0.05$). However, the developmental stages of eggs dealt 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 the control 95.7 d ($P < 0.05$) (Fig. 1).

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	Repellence index after 12 h treatment (%) (±SE)			Repellence index after 24 h treatment (%) (± SE)			Repellence index after 48 h treatment (%) (± SE)		
	300[a]	600	900	300	600	900	300	600	900
C. limonum	71.9 ± 0.1 b[b]	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	41.8 ± 2.9 c	51.4 ± 2.9 c	63.1 ± 3.9 b	35.1 ± 4.5 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	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	59.4 ± 5.3 a	71.2 ± 1.1 a	77.3 ± 3.7 a
M. fragrans	37.7 ± 1.2 c	51.7 ± 2.5 c	59.1 ± 3.0 b	31.6 ± 1.0 c	41.8 ± 1.8 c	47.6 ± 2.8 c	24.3 ± 3.0 b	33.7 ± 2.3 c	41.33 ± 1.3 b
Control	0 d	0 d	0 c	0 d	0 d	0 d	0 c	0 d	0 c
$F_{4,10}$	110.9	173.5	252.0	120.4	183.2	255.8	46.5	171.0	156.2
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[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)	
		48 h	98 h
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	0 d
$F_{4,10}$	—	183.3	344.5
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 (325.3–3,110.4)	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 (280.1–2,263.8)	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 previously 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,

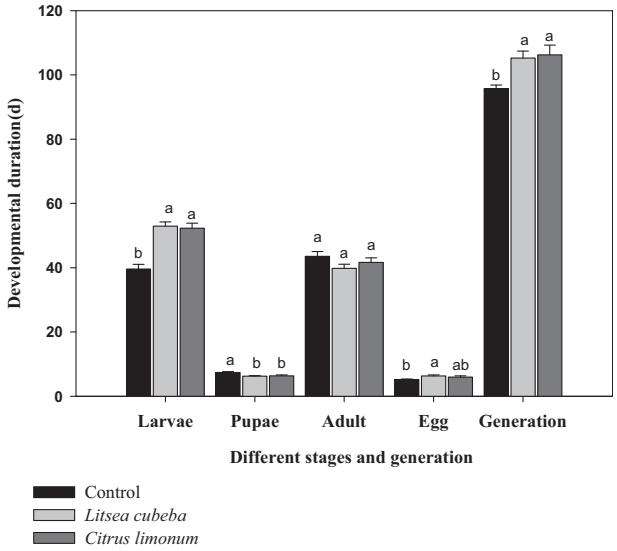

Fig. 1. Effects of EOs on growth and development of larvae, pupae, adult, and egg of *T. molitor*. Note: number of insects tested for each treatment is 90 and the vertical bars represent the standard error of means for three replicates values. The growth and development of larvae, pupae, adult, and egg of *T. molitor* were the avenue of three replications ± SE. Means followed by the same letters do not differ significantly ($P > 0.05$) in the analysis of variance (ANOVA) test. $F2, 6 = 26.15$ and $P < 0.001$ for larvae; $F2, 6 = 7.24$ and $P = 0.03 < 0.05$ for pupae; $F2, 6 = 1.09$ and $P = 0.24, > 0.05$ for adult; $F2, 6 = 3.40$ and $P = 0.10, > 0.05$ for egg; $F2, 6 = 6.76$ and $P = 0.03, < 0.05$ for total growth and development treated with EOs or not.

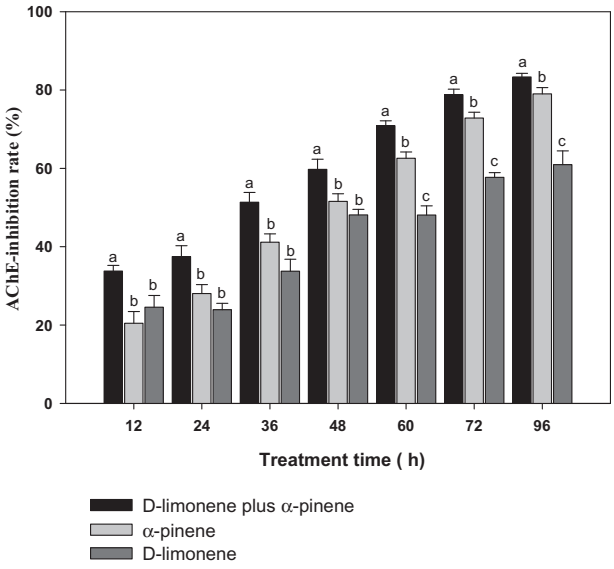

Fig. 2. The time course of the mainly active ingredients of EOs on AChE activity of 10th instar *T. molitor*. Note: number of insects tested for each treatment is 90 and the vertical bars represent the standard error of means for three replicates values. The growth and development of larvae, pupae, adult and egg of *T. molitor* were the avenue of three replications ± SE. Means followed by the same letters do not differ significantly ($P > 0.05$) in the analysis of variance (ANOVA) test. The $F2,6$ values were 6.70, 9.26, 11.75, 8.66, 41.80, 62.55, and 27.14 were at 12, 24, 36, 48, 60, 72, and 96 h, respectively, and all $P < 0.01$.

Table 8. The fumigant toxicity of main ingredients of EOs on 10th instar of *T. molitor*

Treatment	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**
D-Limonene plus β-pinene	48	450	2.9 (2.4–3.5)	48.0 (30.2–92.5)	2.42 ± 0.22	6.69 (4)	< 0.05
	96	450	2.0 (1.6–2.4)	30.7 (20.0–56.1)	2.48 ± 0.23	2.84 (4)	< 0.05
D-limonene	48	450	4.3 (3.6–5.2)	60.1 (39.8–106.6)	2.57 ± 0.23	3.65 (4)	< 0.05
	96	450	3.0 (2.0–4.1)	36.2 (19.7–112.0)	2.72 ± 0.25	6.59 (4)	< 0.05
β-pinene	48	450	10.1 (8.3–12.5)	168.9 (100.4–356.0)	2.41 ± 0.23	1.82 (4)	< 0.05
	96	450	6.5 (5.4–7.8)	94.3 (60.4–175.8)	2.53 ± 0.23	0.83 (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 $P < 0.05$.

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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