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Repellent activity of essential oils extracted from five Artemisia species against *Tribolium castaneum* (Coleoptera: Tenebrionidae)

[Actividad repelente de aceites esenciales extraídos de cinco especies de Artemisia contra *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Abstract: Artemisia genus (family Asteraceae) has been widely used as medicines and cosmetic. The chemical compositions of essential oils extracted from five Artemisia species (*A. anethoides*, *A. giraldii*, *A. roxburghiana*, *A. rubripes* and *A. sacrorum*) were analyzed and the repellent activities of five essential oils were investigated by testing percent repellency (PR) in petri dish against *Tribolium castaneum*. By GC-MS analysis, the common components of the five essential oils were eucalyptol (11.09%-50.05%), camphor (6.28%-33.10%), terpinen-4-ol (2.46%-12.41%), β-caryophyllene (0.63%-10.68%) and germacrene D (2.28%-10.01%). 3,3,6-trimethyl-1,4-heptadien-6-ol (11.72%), 2-isopropyl-5-methyl-3-cyclohexen-1-one (24.80%) and β-farnesene (12.23%) were the characteristic compounds in essential oils of *A. sacrorum*, *A. anethoides* and *A. rubripes* respectively. The essential oils of five plants showed repellent activity against *T. castaneum*. The PR of others four essential oils were comparable with DEET expect for *A. sacrorum*. The results indicated that the essential oils of *A. anethoides*, *A. giraldii*, *A. roxburghiana* and *A. rubripes* had the potential to be developed as repellent for control of *T. castaneum*.

Keywords: Artemisia species, essential oil, *Tribolium castaneum*, repellent activity.

Resumen: El género Artemisia (familia Asteraceae) ha sido ampliamente utilizado como medicamentos y cosméticos. Se analizaron las composiciones químicas de los aceites esenciales extraídos de cinco especies de Artemisia (*A. anethoides*, *A. giraldii*, *A. roxburghiana*, *A. rubripes* y *A. sacrorum*) y se investigaron las actividades repelentes de cinco aceites esenciales mediante la prueba de repelencia porcentual (PR) en placa de petri contra *Tribolium castaneum*. Por análisis GC-MS, los componentes comunes de los cinco aceites esenciales fueron eucaliptol (11,09% -50,05%), alcanfor (6,28% -33,10%), terpinen-4-ol (2,46% -12,41%), β-cariofileno 0,63% -10,68%) y germacrén D (2,28% -10,01%). 3,3,6-trimetil-1,4-heptadien-6-ol (11,72%), 2-isopropil-5-metil-3-ciclohexen-1-ona (24,80%) y β-farneseno (12,23%). Los compuestos característicos en los aceites esenciales de *A. sacrorum*, *A. anethoides* y *A. rubripes* respectivamente. Los aceites esenciales de cinco plantas mostraron actividad repelente contra *T. castaneum*. El PR de otros cuatro aceites esenciales eran comparables con DEET esperado para *A. sacrorum*. Los resultados indicaron que los aceites esenciales de *A. anethoides*, *A. giraldii*, *A. roxburghiana* y *A. rubripes* tienen el potencial de ser desarrollados como repelentes para el control de *T. castaneum*.

Palabras clave: Especies de Artemisia, aceite esencial, *Tribolium castaneum*, actvidad repelente

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INTRODUCTION

Insect repellents produce the vapor barrier that can prevent the pest from approaching objectives (Brown & Hebert, 1997). In ancient times, human have used tars, smokes, plant essential oils and other natural materials to control pests (Peterson & Coats, 2001). Since 20th, synthetic chemical repellents have been used widely to prevent losses of stored grains, fruits and agriculture, which are caused by various pests. *N*,*N*-diethyl-3-methylbenzamide (DEET) is one of the most famous and effective commercial insect repellents (Isman, 2006; Degennaro, 2015). But some literatures have shown that DEET may lead to hypotension, seizures and coma (Antwi & Shama, 2008; Koren *et al*., 2016). And, other synthetic chemical repellents have toxicity to non-target organisms or environment (Sorge, 2009). In addition, almost all repellents can cause the development of resistant pest populations (Evans & Scarisbrick, 1994; Germano *et al*., 2016). These problems urge researchers to find alternative activity substances that are low-toxic and eco-friendly. Botanical repellents are concentrated by researchers for their characters of high safe to health and environment (Pavela, 2007). Botanical repellents are the mixture of secondary metabolites from plants and possess many biologically activities. And, these botanical repellents have shown excellent and eco-friendly insecticidal effect (Isman, 2006; Isman, 2015). The essential oils are one of promising botanical repellents. Many records reported that the essential oils obtained from different plants have repellent activity against stored product pests (Nerio *et al*., 2010). Currently, the US Environmental Protection Agency (US EPA) has registered citronella, lemon and eucalyptus oils as insect repellent agents (Katz *et al*., 2008).

Artemisia spp. (Asteracae family) which includes over 500 species, distribute mainly in Asia, Europe, and North America (Bora & Sharma, 2011). Many plants of this genus contain essential oils and have been used wildly in medicine and cosmetic (Lopes-Lutz *et al*., 2008; Abad *et al*., 2012). The researchers have got the artemisinin from *A.* annua to treat malaria and the artemisinin have saved the lives of countless people (Mannan *et al.,* 2010). Some essential oils of *Artemisia* spp. plants have shown definite insecticidal and repellent activity against many species of pests. For example, *A. giraldii* and *A. rubripes* showed contact and fumigant activity against *Tribolium castaneum* and *Lasioderma serricorne* (Liang *et al.,* 2016); *A. rupestris* showed repellent and contact activity against *Liposcelis bostrychophila* (Liu *et al.,* 2013); *A. anethoides* possessed contact, fumigant and repellent activity against *T. castaneum* and *L. serricorne* (Liang *et al.*, 2017); *A. vulgaris* exhibited repellent and fumigant activity to *T. castaneum* (Wang *et al.,* 2006); *A. sieberi* showed fumigant toxicity to three pests of *Callosobruchus maculatus, Sitophilus oryzae* and *T. castaneum* (Negahban *et al.,* 2007); *A. princeps* possessed repellent and insecticidal activities to *S. oryzae* and *Bruchus rugimanus* (Liu *et al.*, 2006); *A. annua* showed repellency and toxicity to *T. castaneum* and *Callosobruchus maculatus* (Tripathi *et al.,* 2000); fumigant and contact toxicities of *A. lavandulaefolia* and *A. sieversiana* to *S. zeamais* were obvious (Liu *et al.*, 2010); *A. herba-alba* and *A. absinthium* exhibited fumigant and contact toxicities against *Orysaephilus surinamensis* and *T. castaneum* (Bachrouch *et al.,* 2015); *A. mongolica*, *A. argyi* and *A. stolonifera* showed fumigant, contact and repellent activity against *L. serricorne* (Zhang *et al.,* 2014; You *et al.*, 2015; Zhang *et al.*, 2015).

The red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) lived the stored products, such as stored grains. And, red flour beetle can survive in extremely dry environments (Saroukolai *et al*., 2010). *T. castaneum* have resulted in serious economic loss and decrease in nutritional value (Khan *et al*., 2015). It has demonstrated that this beetle has resistance to many classes of insecticides (Hoppe, 2009). In this work, the repellent activity of the essential oils extract from five species of *Artemisia* spp. plants (*A. anethoides*, *A. giraldii*, *A. roxburghiana*, *A. rubripes* and *A. sacrorum*) was investigated. It is expected to find new materials for controlling *Tribolium castaneum*.

MATERIAL AND METHODS

Plant materials

The vegetable materials from five species of plants (Table 1) were obtained from China in 2015. The species were identified by Dr. Liu, Q.R. and the voucher specimen of five plants was deposited at the College of Resources Science and Technology, Beijing Normal University of China in October 2015.

Insects

T. castaneum of been used in all experiments were reared in the Beijing Key Laboratory of Traditional Chinese Medicine Protection and Utilization, College of Resources Science and Technology, Beijing Normal University, China. The insects were maintained in 0.5 L glass jar containing food medium of wheat flour (passed through a 250 micrometer aperture sieve) mixed with yeast (10:1, w/w). The jar was placed in constant temperature oven, and the culture condition is 30 ± 1º C and 70–80% relative humidity (rh). The unsexed insects in all of the experiments were 1-2 weeks old post-eclosion adults.

Extraction and analysis of essential oil

The aerial parts of the five plants were dried in shade place rapidly. The dried samples (200 g of each plant) were powdered and subjected to hydrodistillation for 5 h by using a Clevenger-type apparatus (3000 mL). The crude essential oils were obtained by removing lower level of water. Then crude essential oils were dried by using anhydrous Na_2SO_4. The volumes of pure essential oils were measured and their yields (the volume of the essential oil divided by the mass of the dry plant) and density (the mass of the essential oil divided by the mass of water under the same volume) were calculated. The essential oil was stored in airtight containers in refrigerator at 4º C. The analysis of the major chemical components of the essential oil samples was carried out in Agilent 6890N gas chromatograph using a flame ionization detector (FID) and coupled to Agilent 5973N mass spectrometer. A capillary column HP-5MS (30 m × 0.25 mm × 0.25 μm) was used. The chromatographic conditions were followed as: initial column temperature 60º C, keeping 1 min, then heated up from 60 to 180º C (10º C/min, keeping 1 min), 180 to 280º C (20º C /min, keeping 5 min). 1 μL sample (dilute to 1% with hexane) was injected into gas chromatograph. The injector temperature was maintained at 270º C, split ratio 1:10, carrier gas was helium, flow rate 1.0 mL/min. The mass spectrometer conditions were: ionization energy 70 eV, mass range 50–550 *m/z*.

The identification of the components were based on comparison of their retention indices (RI) obtained using n-alkanes (C_8–C_{24}) under the same operating conditions. On the other hand depended on their mass spectra with those stored in NIST 05 (Standard Reference Data, Gaithersburg, MD, USA) and Wiley 275 libraries (Wiley, New York, NY, USA) or with mass spectra from the literature (Adams, 2001). Relative percentages of the individual constituents of the essential oil were obtained by averaging the GC-FID peak area% reports.

Repellent activity bioassay

The repellent activity of the essential oils against *T. castaneum* was assessed based on the method (Zhang *et al*., 2011). The essential oils of five plants respectively were dissolved in *n*-hexane to prepare serials of testing solutions with five concentrations (78.63, 15.73, 3.15, 0.63 and 0.13 nL/cm^2). Petri dishes (9 cm in diameter) were used as experimental equipment. A filter paper (9 cm in diameter) was cut into two equal pieces. 500 μL of testing solution was uniformly painted on one piece as a testing part, while another piece was treated with 500 μL of *n*-hexane as a negative control part. After they were air dried for 30 s to evaporate the solvent completely, the two pieces of filter paper were fixed on the bottom of a Petri dish side by side. For each test, twenty insects were placed at the center of the disk which was then covered with a lid. Five replicates were performed in all tests. DEET was used as the positive control. Numbers of the insects present on each strip were counted after 2 h and 4 h. The value of percent repellency (PR) was calculated with following equation:

$$PR (\%) = [(Nc-Nt) / (Nc+Nt)] \times 100$$

here: Nc was the number of insects present on the negative control half, Nt was the number of insects present on the testing part, and then the averaged PR values and the standard error (SE) values were derived by IBM SPSS V22.0.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The yield and density of essential oils were listed in Table 1. The main components of five essential oils were showed in Table 2. The components of the five essential oils had similarity and diversity. For example, eucalyptol (11.09%-50.05%), camphor (6.28%-33.10%), terpinen-4-ol (2.46%-12.41), β-caryophyllene (0.63%-10.68%) and germacrene D (2.28%-10.01%) were almost identified in every essential oil, but the relative contents of these compounds were various in different essential oils. The reason may be that the five essential oils were extracted from the plants of common genus. Eucalyptol, camphor and terpinen-4-ol have been found in other *Artemisia* spp. plants. For example, above the three compounds were found in the essential oil of *A. argyi*, *A. stolonifera* and *A. mongolica*, and theirs percentage composition were more higher than other compounds (Zhang *et al*., 2014; You *et al*., 2015; Zhang *et al*., 2015). Despite of similarity, the components of five essential oils had obvious differences. Some components were identified only in certain plant. For example, 3,3,6-trimethyl-1,4-heptadien-6-ol (11.72%), 2-isopropyl-5-methyl-3-cyclohexen-1-one (24.80%) and β-farnesene (12.23%) were the characteristic compounds in essential oils of *A. sacrorum*, *A. anethoides* and *A. rubripes* respectively. In previous reports (Khanina *et al*., 1991; Mathela *et al*., 1994; Chen, 2000; Haider *et al*., 2009; Lohani *et al*., 2009; Chu *et al*., 2012; Xia *et al*., 2014; Bicchi *et al*., 2015; Liang *et al*., 2016; Liang *et al*., 2017), the main chemical compounds of essential oils extracted from five *Artemisia* spp. plants (*A. anethoides, A. giraldii, A. roxburghiana, A. rubripes and A. sacrorum*) were eucalyptol (8.13% - 40.72%), camphor (15.2% - 22.50%), germacrene (2.28%- 5.68%), thujone (12.0% - 65.3%) and caryophyllene (13.85% - 18.4%). Meanwhile, the some essential oils contained limonene (19.67%), eugenol (16.2%), camphene (14.09%), β-pinene (13.18%), iso-elemicin (10.08%). The components of five essential oils in this work were not exactly same with previous reports. These differences might arise from collection time, location and altitude factors, as well as extraction methods, and these diversities may result in different biological activities.

Table 1
Collection data of the plants and yields of essential oils extracted from plants

Plant species	Collected time	Collect location	Coordinate & altitude (m)	Part used	Yield (%)	Density (g/ml)
A. giraldii	2015.07	Tianshui city	34°34′03″N 105°42′58″E 1244	aerial part	0.45	0.98
A. roxburghiana	2015.07	Tanchang county	33°58′01″N 104°25′15″E 1776	leaf	0.50	0.96
A. rubripes	2015.06	Yongdeng county	36°40′18″N 102°33′12″E 1900	aerial part	0.83	0.97
A. sacrorum	2015.07	Tanchang county	33°58′01″N 104°25′15″E 1776	leaf	0.70	0.98
A. anethoides	2015.08	Lintan county	34°58′18″N 103°39′12″E 2233	aerial part	0.05	0.97

Repellent activity

The essential oils of five species of plants exhibited certain repellent activity against *T. castaneum* (Figure. 1). At 2 h after exposure, the PR of five essential oils were higher than 90% at testing concentration of 78.63 nL/cm^2, and the result was same with positive control DEET. The PR of *A. roxburghiana, A. rubripes and A. anethoides* were 100% at lower testing concentration of 15.73 nL/cm^2. The PR of *A. rubripes, A. sacrorum and A. anethoides* were same with DEET in testing concentration of 3.15, 0.63 and 0.13 nL/cm^2, even in the lowest testing concentration of 0.13 nL/cm^2, the PR of *A. rubripes, A. sacrorum and A. anethoides* obvious were higher than DEET.

At 4 h after exposure, the PR results of five essential oils were different with 2 h after exposure. For example, the PR of *A. rubripes* and *A. anethoides* were higher than DEET in testing concentration of 3.15, 0.63 nL/cm^2 and only *A. rubripes* was higher than DEET in testing concentration of 0.63 nL/cm^2. The PR of *A. rubripes* obvious was higher than DEET in the lowest testing concentration of 0.13 nL/cm^2. So the essential oil of *A. rubripes* showed outstanding repellent activity among five essential oils.

Table 2
The main chemical components of the essential oils extracted from five plants

compound	RT*	RI**	Relative content (%)				
			A. giraldii	*A. roxburghiana*	*A. rubripes*	*A. sacrorum*	*A. anethoides*
3,3,6-Trimethyl-1,4-heptadien-6-ol	3.64	917				11.72	
Z-2,7-dimethyl-4-Octene-2,7-diol	3.97	967					8.23
Terpin hydrate	4.03	980					27.03
Eucalyptol	4.06	1017	40.72	50.05	11.09	16.96	
Artemisia ketone	4.24	1062				2.29	
3,3,6-Trimethyl-1,5-heptadien-4-ol	4.44	1073				4.42	
β-Terpineol	4.69	1088	1.59	2.63	0.76		4.89
Thujone	4.74	1114			1.708	4.56	
cis-p-Menth-2-en-1-ol	4.90	1118	1.97			1.44	1.23
Camphor	5.13	1120	22.50	33.10	7.01	14.46	6.28
α-Pinocarvone	5.26	1147			3.79		1.96
Borneol	5.35	1159			2.94	3.08	
Terpinen-4-ol	5.40	1172	12.41	7.09	2.46	2.94	
4-Terpineol	5.42	1177					4.11
α-Terpineol	5.52	1184	3.60			1.92	1.50
α-Fenchene	5.53	1185		2.10			
2-isopropyl-5-methyl-3-Cyclohexen-	6.02	1250					24.80
β-Caryophyllene	7.29	1414	1.36	0.63	10.68	5.43	1.50
β-Farnesene	7.41	1450			12.23		
Di-epi-α-cedrene-(I)	7.65	1467			5.73		
α-Curcumene	7.68	1472			4.64		
Germacrene D	7.75	1479	2.28	3.00	10.01	2.36	3.35
β-Selinene	7.83	1482				1.97	
γ-Elemene	7.86	1484					2.10
δ-Cadinene	7.99	1510			2.60		
4-(methylthio)-Phenol	8.09	1527					2.64
Davanone	8.51	1563			2.80		7.27
Caryophyllene oxide	8.71	1566	0.32		1.48	1.63	
8-Cedren-13-ol	9.25	1581	4.15				
(-)-T-Muurolol	9.34	1610			1.94		
Longiverbenone	9.50	1617				8.52	
β-Eudesmol	9.56	1625				5.05	
γ-Gurjunene	9.60	1631				2.89	

***RT, retention time;**
**** RI, retention index as determined on a HP-5MS column using the homologous series of n-alkanes**

There was different PR value between five essential oils against T. castaneum. The essential oil of A. giraldii showed 100% PR at test concentration of 78.63 nL/cm2. But at lower testing concentration, it's PR decreased obviously compared with other essential oils. As a whole, the PR was proportional to the concentration of the essential oils. Expect for A. sacrorum, the PR of other essential oils were comparable with DEET at five testing concentration at 2 h and 4 h after exposure. Especially, essential oil of A. rubripes showed outstanding repellent activity among five plants for its higher PR than DEET at all test concentration at 2 h and 4 h after exposure.

There were significant differences of repellent activity between five essential oils and DEET ($F = 29.32$, $df = 5$, $P < 0.01$), the sequence of repellent activity was *A. rubripes* > *A. anethoides* > *A. roxburghiana* = *A. sacrorum* = DEET > *A. giraldii*. Meanwhile, there were significant differences between five concentrations ($F = 299.43$, $df = 4$, $P < 0.01$), and the repellent activity was added along concentration increased. About exposure time, there were significant differences between 2h and 4h ($T = 2.67$, $df = 296$, $P < 0.05$), and the repellent activity of 2h exposure was significant higher than 4h exposure.

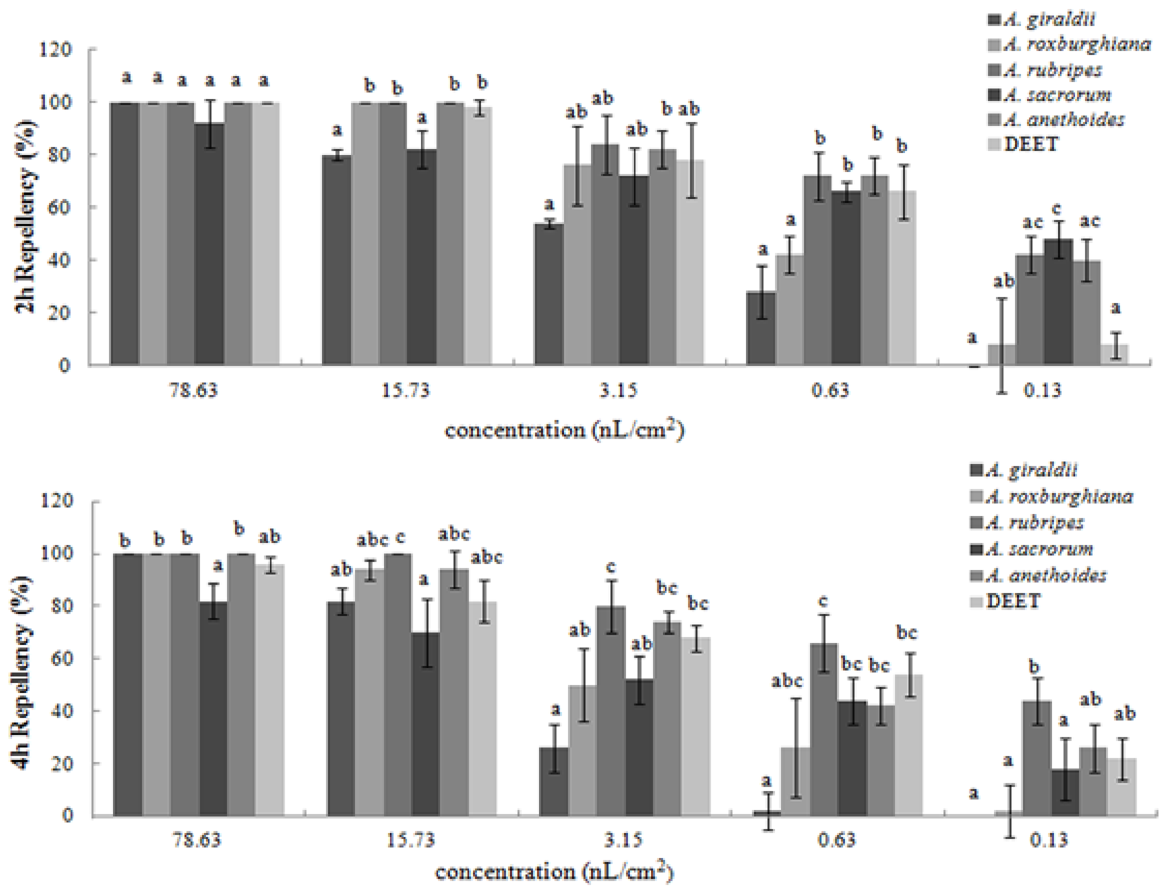

Figure 1
Percentage repellency (PR) of the essential oil extracted from five *Artemisia* spp. plants against *T. castaneum* at 2 h and 4 h after exposure
[a,b,c] means in the same column followed by the same letters do not differ significantly ($P > 0.05$) in ANOVA and Tukey's tests. PR was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests.

The previous literature (Zhang *et al*., 2015) showed that the main components (eucalyptol, camphor and terpinen-4-ol) of five essential oils possess certain repellent activity at test concentration (range from 0.06 to 39.32 nL/cm^2) against *T. castaneum*. So the repellent activity of essential oils could be related to the existence of such chemical compounds. And the literature showed terpinen-4-ol possessed higher repellent activity than eucalyptol and camphor at test concentration. But the content of terpinen-4-ol in the essential oil of *A. rubripes* (2.46%) was lower than *A. giraldii*, *A. roxburghiana* and *A. sacrorum*. So the repellent activity of five essential oils did not due to single compound, it may be attributed to synergistic effect of multiple compounds.

CONCLUSIONS

The essential oils extracted from five *Artemisia* spp. plants showed repellent activity against *T. castaneum*. Thus, these essential oils have potential to develop into new natural pest repellents. It was same with previous reports, the essential oils of *Artemisia* spp. plants possessed obvious repellent activity against *T. castaneum* and other pests. So *Artemisia* spp. plants were very suitable resources of developing into repellent agents against pests. Some repellents containing DEET caused side effects. The researchers have evaluated various repellent ingredients and found plant essential oil is alternative repellent agents, and it is easy to volatilize in air and degrade in nature. There are few reports about side effects of plant essential oil to the human body. Thus, repellent agents based on essential oils are receiving increased attention worldwide Although repellent effect of essential oils was comparable or even better than those of synthetics chemical repellents, its high volatility decreased action time (Fradin & Day, 2002). The research about the release of essential oils has many records. For example, some literatures showed that microcapsule method can extend the release time of essential oils greatly (Banerjee *et al*., 2013; Liu *et al*., 2012). The natural insecticides and repellents used in pest control are concerned by researchers for low toxicity. However, these natural insecticides are not always more safer than synthetic chemical repellents. Most of them have not been evaluated for effectiveness and safety (Trumble, 2002; Foster *et al.*, 2005). The components of the essential oils extracted from same species of plant were various due to the difference in collecting time, location and altitude. Thus the pest control measures based on essential oils were unstable. So some standards must be built to ensure that the repellents effect of essential oils were stable in practical application. For example, collection time and location of aromatic plants must be confirmed, and the extract method also should be uniform.

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