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SHORT RESEARCH AND DISCUSSION ARTICLE

Toxicities of monoterpenes against housefly, *Musca domestica* L. (Diptera: Muscidae)

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Abstract The development of natural plant extracts and essential oils will assist to decrease the negative effects of synthetic chemicals. Many plant extracts and essential oils contain monoterpenes, sesquiterpenes, and aliphatic compounds. In the present study, the fumigation activity of 42 pure monoterpenes against housefly, *Musca domestica*, was evaluated. Results from fumigation tests revealed that ρ-cymene, terpinolene, (±)-menthol, thymol, carvacrol, (−)-carvone, (+)-camphor, (+)-pulegone, (−)-menthone, citral, (±)-citronellal, cuminaldehyde, and citronellyl acetate exhibited strong fumigation activity against *M. domestica*. Specifically, the compounds of (+)-pulegone, cuminaldehyde, citral, and ρ-cymene had a highest toxicity toward *M. domestica* with LC_{50} values of 0.26, 0.60, 0.64, and 0.77 μl/L, respectively. The present results indicated that (+)-pulegone, cuminaldehyde, citral, and ρ-cymene are promising toxicants against *M. domestica* and could be useful in the search for new natural insecticides.

Keywords *Musca domestica* · Monoterpenes · Fumigant · (+)-Pulegone · Cuminaldehyde

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Introduction

The housefly, *Musca domestica* L. (Diptera: Muscidae), as the carrier of more than 100 human and animal intestinal diseases, is one of the most common insect in human settlements (Malik et al. 2007; Kumar et al. 2012a, b, c, 2013, 2014). Traditionally, the control of housefly is usually accomplished by using chemical insecticides, such as organochlorines, organophosphates, and pyrethroids, but long-term use of chemical insecticides has been showing significant effect on human health and environmental pollution (Kumar et al. 2012a). These problems have been called for the urgent need for new strategies of housefly control. Therefore, the search for natural bioactive compounds from plants as alternative to synthetic insecticide is becoming much essential (Kumar et al. 2011; Mishra et al. 2011; Pavela 2013).

Many plant extracts and essential oils are believed to have insecticidal properties, and numerous plant extracts and essential oils have been extensively studied against various insect species (Kordali et al. 2006; Cheng et al. 2009; Palacios et al. 2009a,b; Xie et al. 2013, 2014a, 2015; Bougherra et al. 2015; Mansour et al. 2015; Peixoto et al. 2015). Besides, the plant extracts and essential oils had been reported to have the insecticidal activity against the housefly (Kumar et al. 2011, 2012a, b, c, 2013). Futhermore, the monoterpenes had been detected to possess the insecticidal activity against the housefly and, in general, menthol, limonene, citral, 1,8-cineole, menthone, and pulegone were found to be more toxic (Rice and Coats 1994; Lee et al. 2003; Palacios et al. 2009a, b; Kumar et al. 2013, 2014). In addition, Zhang et al. (2016) showed that (±)-citronellal and (+)-pulegone were effective fumigants against *Drosophila melanogaster*. Now, we are interesting to know whether monoterpenes possess the insecticidal property against housefly. However, to the best of our knowledge, no studies were conducted to systematic evaluate the fumigant toxicity of monoterpenes toward *M. domestica*. Therefore, this paper describes a laboratory study to examine the toxicity of 42 monoterpenes against *M. domestica*.

Materials and methods

Materials

All experimental monoterpenes compounds were purchased from commercial sources, part of compounds were purchased from Sigma-Aldrich Chemical (Shanghai, China), and the others were purchased from Tokyo chemical Industry Co., Ltd. (Shanghai, China), and were of 95% purity or greater. Chemical structures for the tested compounds are provided in Xie et al. (2014b). The positive control insecticide dichlorvos (DDVP, 98%) was purchased from LHDUBANG Agrochemicals Co., Ltd. (Shanghai, China). Insecticide stock solutions were prepared at a standard concentration of 100 mg/mL in analytical-grade acetone. For liquid monoterpenes compounds, directly collected a certain amount for experimental study. Stock solutions were stored at − 20 °C in sealed amber-colored vials.

Housefly

Adult houseflies collected from field by sweep net method were reared in insect rearing cages contained a diet of milk powder and wheat bran, according to the method described by Kumar et al. (2011). Hatched larvae were transferred to plastic basin (25 × 18.5 cm^2) contained a larval diet (wheat bran) which was changed daily until larvae reached the pupal stage.

Fumigation assay

The fumigation bioassay method of Zhang et al. (2016) was employed to assess the insecticide activity of 42 volatile compounds. A filter paper (Whatman No 1 cut into 2 cm diameter pieces) was impregnated with respective dosages of compounds and then attached to the undersurface of the 1000 mL glass jar's (10 cm diameter × 12.5 cm) screw cap, respectively. The cap was tightly screwed onto the jar, which contained 20 houseflies. Adults used in all experiments were of unknown age and sex. Jars with the untreated filter paper (contained acetone) were considered as controls. Three replicates of each control and treatment were set up. After 24 h, mortality was scored with the aid of a magnifying glass. Flies were considered dead only when they showed a complete lack of movement. All bioassays were conducted at room temperature (23–25 °C) under atmospheric pressure conditions.

Statistical analyses

Probit analysis of concentration-mortality data were performed using PROC PROBIT in the SPSS version 19.0 software package (SPSS Inc., Chicago, USA). LC_{50}s were considered significantly different using the criterion of nonoverlap of 95% confidence intervals (CIs).

Results

To evaluate the toxicities of 42 pure commercial monoterpenes, including 11 hydrocarbons, 12 alcohols, 4 phenols, 6 ketones, 3 aldehydes, and 6 acetate esters, were determined against adults of *M. domestica*. Among the monoterpene hydrocarbons, ρ-cymene, α-terpinene, γ-terpinene, and terpinolene were more toxic against the housefly, with 100% mortality against the housefly at a concentration of 5 μl/L (Fig. 1a). Among the tested alcohols, the weak toxicity against the adult housefly was shown by (−)-borneol, nerol, geraniol, and 3,7-dimethyl-1-octanol (Fig. 1b). The toxicities of eugenol and isoeugenol were less than 30% at a concentration of 5 μl/L (Fig. 1c), and the ketones, aldehyde, and acetate esters possessed significant fumigant activities (mortality > 80%) against adult housefly at a concentration of 5 μl/L (Fig. 1d, e, f).

The LC_{50} values of the 42 monoterpenes against of *M. domestica* were determined and shown in Table 1. The insecticidal activity of (+)-pulegone, cuminaldehyde, citral, and ρ-cymene had a higher toxicity toward *M. domestica* with LC_{50} values of 0.26, 0.60, 0.64, and 0.77 μl/L, respectively. With the exception of the positive control insecticides, all other tested compounds had LC_{50} estimates ranging from 0.26 to 37.50 μl/L. The positive control DDVP showed considerably higher toxicity than any of the monoterpene compounds (LC_{50} = 0.001 μl/L). In hydrocarbon monoterpenes, with the exception of ρ-cymene (LC_{50} = 0.77 μl/L) and terpinolene (LC_{50} = 1.84 μl/L), each other compounds of hydrocarbon group exhibited weak insecticidal activity. The aldehydes cuminaldehyde, citral, and (±)-citronellal had pronounced toxicity with LC_{50} values of 0.60, 0.74, and 1.84 μl/L, respectively. The alcohol compounds, (±)-menthol, (±)-terpinen-4-ol, and isopulegol showed the strongest effect against *M. domestica* (LC_{50} = 1.38, 2.03 and 2.81 μl/L, respectively), followed by 1,8-cineole, α-terpineol, linalool, geraniol, dihydrolinalool, β-citronellol, (−)-borneol, nerol, and 3,7-dimethyl-1-octanol with LC_{50} values of 3.38, 3.74, 4.43, 4.69, 5.38, 5.50, 7.73, 10.34, and 10.70 μl/L in 24 h, respectively. There was no significant difference in the insecticidal activity of members in the alcohol group. In a test with phenols, the insecticidal activities of thymol (LC_{50} = 1.60 μl/L) and carvacrol (LC_{50} = 1.69 μl/L) were higher than eugenol (LC_{50} = 6.94 μl/L) and isoeugenol (LC_{50} = 37.50 μl/L). The ketone compounds showed strong insecticidal activity against

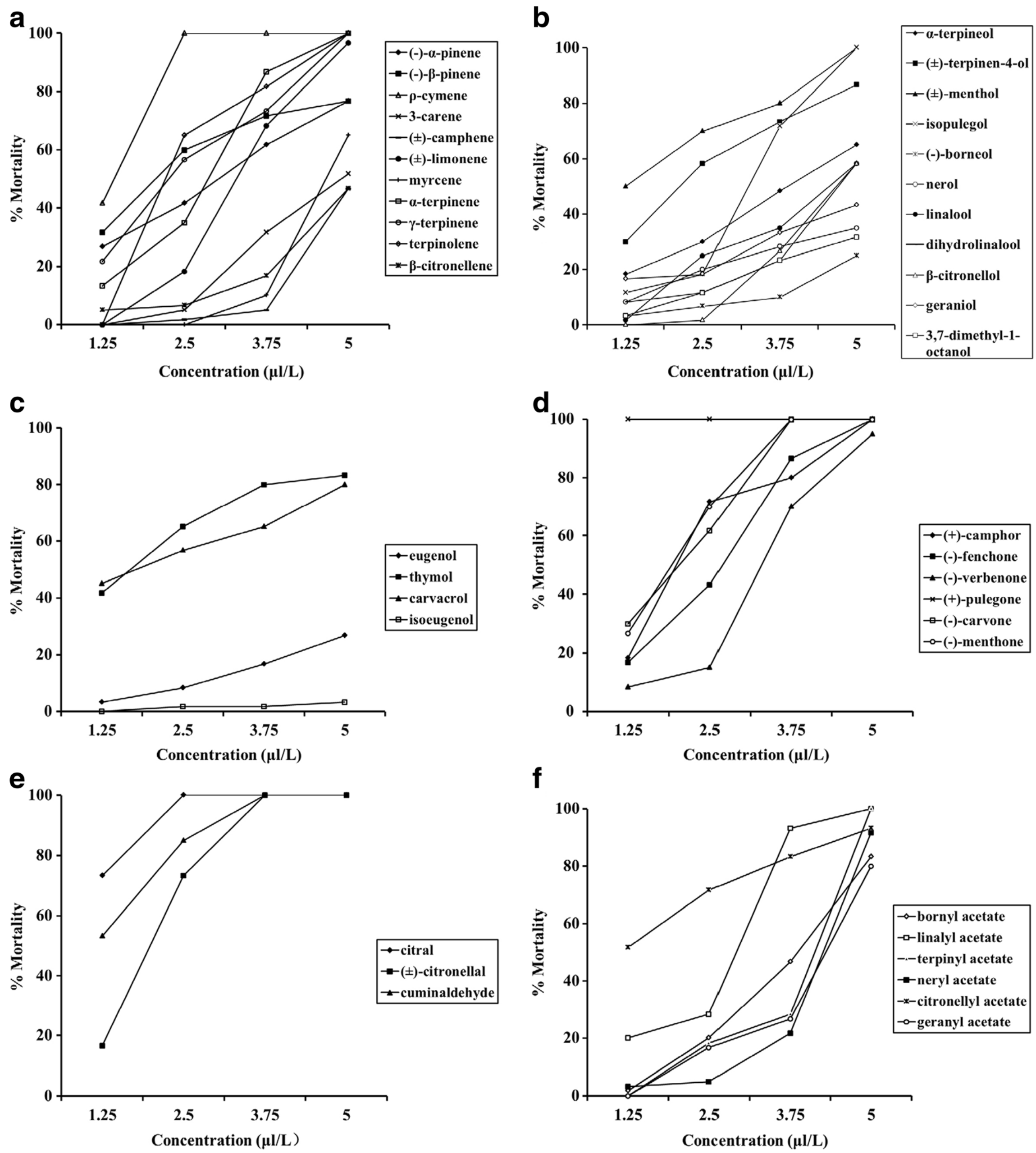

Fig. 1 Toxicity of 42 monoterpenes (**a** hydrocarbons; **b** alcohols; **c** phenols; **d** ketones; **e** aldehydes; **f** acetate esters) against adults of the housefly, *Musca domestica*. Means ($N = 3$) using 20 housefly per replicate

M. domestica. Among these ketone compounds, (+)-pulegone demonstrated the best *M. domestica* toxicity effect at 24 h with LC_{50} value of 0.26 μl/L, followed by (−)-menthone ($LC_{50} = 1.73$ μl/L), (−)-carvone ($LC_{50} = 1.76$ μl/L), (+)-camphor ($LC_{50} = 1.99$ μl/L), (−)-fenchone ($LC_{50} = 2.26$ μl/L), and (−)-verbenone ($LC_{50} = 3.01$ μl/L), respectively. Citronellyl acetate ($LC_{50} = 1.26$ μl/L) exhibited the strongest insecticidal activity among the acetate esters group, followed by linalyl acetate ($LC_{50} = 3.30$ μl/L), bornyl acetate ($LC_{50} = 3.57$ μl/L), terpinyl acetate ($LC_{50} = 3.83$ μl/L), neryl acetate ($LC_{50} = 4.16$ μl/L), and geranyl acetate ($LC_{50} = 4.24$ μl/L), respectively.

Discussion

The present results showed that the 42 monoterpenes tested had varying degrees of insecticidal activity against adult housefly, and the mortality generally increased with increasing doses of the monoterpenes. Among the tested compounds, (+)-pulegone, cuminaldehyde, citral, and ρ-cymene showed relatively strong toxicity against adult housefly.

In recent years, the fumigation toxicity of some monoterpenes against the various insect species were reported in several studies (Rice and Coats 1994; Lee et al. 2003;

Table 1 LC_{50} and LC_{90} values (μl/L) of 42 monoterpenes against adults of the housefly, *Musca domestica* in 24 h

Compound	Slope (±SE)[a]	LC_{50} (95% CI)[b]	LC_{90} (95% CI)[b]	χ^{2c}	P^{c}
Hydrocarbons					
(−)-α-pinene	2.20 (± 0.39)	2.65 (2.19–3.17)	10.13 (7.05–20.56)	3.70	0.96
(−)-β-pinene	2.04 (± 0.38)	2.04 (1.54–2.46)	8.62 (6.07–17.51)	2.33	0.99
ρ-cymene	2.53 (± 0.28)	0.77 (0.59–1.01)	2.48 (1.71–4.76)	17.53	0.06
3-carene	4.63 (± 0.52)	5.02 (4.57–5.56)	9.49 (8.12–11.94)	13.43	0.20
(±)-camphene	5.20 (± 0.61)	6.15 (5.67–6.68)	10.85 (9.55–13.13)	13.39	0.20
(±)-limonene	8.70 (± 0.98)	3.22 (3.02–3.42)	4.52 (4.18–5.05)	7.86	0.64
myrcene	8.10 (± 0.89)	4.95 (4.49–5.42)	7.16 (6.41–8.60)	16.03	0.10
α-terpinene	5.22 (± 0.54)	2.41 (2.03–2.80)	4.25 (3.56–5.72)	21.23	0.02
γ-terpinene	3.86 (± 0.45)	2.15 (1.89–2.40)	4.61 (3.97–5.71)	11.48	0.32
terpinolene	3.69 (± 0.45)	1.84 (1.58–2.08)	4.09 (3.52–5.08)	7.21	0.71
β-citronellene	3.25 (± 0.42)	5.91 (4.94–7.78)	14.63 (10.22–30.53)	22.35	0.05
Alcohols					
α-Terpineol	2.13 (± 0.28)	3.74 (3.16–4.44)	14.99 (10.82–25.40)	5.95	0.95
(±)-Terpinen-4-ol	2.61 (± 0.40)	2.03 (1.66–2.38)	6.30 (4.95–9.52)	7.76	0.65
(±)-Menthol	2.61 (± 0.43)	1.38 (0.80–1.80)	4.28 (3.25–7.67)	15.60	0.11
Isopulegol	5.06 (± 0.54)	2.81 (2.26–3.44)	5.04 (3.99–8.23)	34.43	0.001
(−)-borneol	3.49 (± 0.46)	7.73 (6.73–9.32)	17.98 (13.72–28.0)	4.72	0.91
Nerol	1.45 (± 0.20)	10.34 (8.08–14.53)	79.33 (43.89–114.08)	8.54	0.93
Linalool	3.44 (± 0.55)	4.43 (3.89–5.34)	10.45 (7.84–17.76)	5.31	0.87
Dihydrolinalool	2.73 (± 0.49)	5.38 (4.21–9.38)	15.90 (9.20–43.61)	14.91	0.14
β-Citronellol	4.12 (± 0.45)	5.50 (4.97–6.17)	11.26 (9.45–14.54)	17.51	0.18
Geraniol	2.59 (± 0.25)	4.69 (3.93–5.64)	14.67 (11.06–22.63)	23.56	0.10
3,7-Dimethyl-1-octanol	1.78 (± 0.39)	10.70 (8.64–14.10)	56.36 (31.99–80.76)	4.46	0.92
Phenols					
Eugenol	3.31 (± 0.30)	6.94 (5.93–8.33)	16.94 (13.15–24.79)	24.32	0.08
Thymol	2.47 (± 0.40)	1.60 (1.20–1.93)	5.30 (4.20–7.95)	5.51	0.86
Carvacrol	1.47 (± 0.29)	1.69 (1.03–2.22)	12.55 (8.12–31.61)	7.01	0.90
Isoeugenol	3.29 (± 0.36)	37.50 (32.69–43.45)	92.05 (74.05–126.10)	8.18	0.61
Ketones					
(+)-Camphor	4.39 (± 0.49)	1.99 (1.65–2.31)	3.90 (3.29–5.11)	15.14	0.13
(−)-Fenchone	4.84 (± 0.51)	2.26 (1.94–2.58)	4.16 (3.54–5.36)	15.16	0.13
(−)-Verbenone	5.25 (± 0.57)	3.01 (2.53–3.56)	5.29 (4.31–7.83)	25.81	0.004
(+)-Pulegone	2.55 (± 0.31)	0.26 (0.19–0.33)	0.83 (0.58–1.59)	17.05	0.07
(−)-Carvone	4.83 (± 0.55)	1.76 (1.45–2.04)	3.24 (2.73–4.26)	16.66	0.08
(−)-Menthone	5.31 (± 0.60)	1.73 (1.55–1.91)	3.02 (2.69–3.54)	9.14	0.52
Aldehydes					
Citral	3.43 (± 0.34)	0.74 (0.64–0.85)	1.74 (1.43–2.27)	12.08	0.28
(±)-Citronellal	6.34 (± 0.68)	1.84 (1.67–2.01)	2.94 (2.65–3.38)	4.74	0.91
Cuminaldehyde	3.05 (± 0.32)	0.60 (0.51–0.70)	1.57 (1.27–2.11)	13.89	0.18
Acetates					
Bornyl acetate	5.56 (± 0.62)	3.57 (3.29–0.87)	6.07 (5.38–7.21)	12.20	0.51
Linalyl acetate	4.87 (± 0.51)	2.30 (1.79–2.83)	4.21 (3.33–6.77)	35.49	0.00
Terpinyl acetate	6.90 (± 0.87)	3.83 (3.22–4.49)	5.88 (4.87–10.56)	41.40	0.00
Neryl acetate	10.03 (± 1.52)	4.16 (3.82–4.51)	5.58 (5.02–7.01)	22.56	0.01
Citronellyl acetate	2.21 (± 0.41)	1.26 (0.81–1.61)	4.79 (3.74–7.52)	3.69	0.96
Geranyl acetate	4.84 (± 0.52)	4.24 (3.89–4.62)	7.80 (6.83–9.43)	17.63	0.17
Oxides					
1,8-Cineole	9.92 (± 1.16)	3.38 (3.19–3.57)	4.55 (4.24–5.04)	10.58	0.39
DDVP	3.29 (± 0.46)	0.001 (0.001–0.001)	0.002 (0.002–0.003)	13.00	0.22

[a] Slope ± SE of the probit mortality line

[b] LC_{50} or LC_{90} values (with 95% confidence intervals) in microliter per assay jar (=μl/liters)

[c] Pearson χ^2 statistic with *P* values indicating goodness-of-fit for data to the expected probit response model

Kordali et al. 2007; Abdelgaleil et al. 2009; Cheng et al. 2009; Abdelgaleil 2010; Santos et al. 2011; Kumar et al. 2014; Xie et al. 2014b; Zhang et al. 2016). Previously, monoterpenes were found to possess the varying insecticidal activities on the various insect species and, in general, limonene, (+)-pulegone, menthone, terpinen-4-ol, menthol, α-pinene, fenchone, carvone, cuminaldehyde, 1,8-cineole, myrcene, and carvacrol were found to be more toxic (Prates et al. 1998; Kim and Ahn 2001; Park et al. 2003; Lee et al. 2003; Papachristos et al. 2004; Kordali et al. 2007; Samarasekera et al. 2008; Abdelgaleil et al. 2009; Abdelgaleil 2010; Santos et al. 2011; Xie et al. 2014b). Similarly, it has been found that the monoterpenes possessed the insecticidal activities against the housefly and, in general, menthol, limonene, citral, 1,8-cineole, menthone, and pulegone were found to be more toxic (Rice and Coats 1994; Lee et al. 2003; Palacios et al. 2009a,b; Kumar et al. 2013, 2014). In addition, Zhang et al. (2016) showed that thymol, carvacrol, citral, (±)-citronellal, cuminaldehyde, and (+)-pulegone were effective fumigants against *Drosophila melanogaster*. However, no systematic report was found in the literature on the toxic effects of monoterpenes against housefly. In this respect, this paper is a first systematic report on the toxicities of monoterpenes to *M. domestica*. The results in the present study showed that these compounds were also more effective against the housefly. From the results of the present study, it is expected that monoterpenes could be used successfully as control agent to the *M. domestica*.

In our study, eugenol exhibited weak insecticidal activity against the housefly, the LC_{50} value of eugenol against *M. domestica* was 6.94 μl/L. These results agreed with those of Huang et al. (2002), who also reported the less toxic activity of eugenol against *Sitophilus zeamais* and *Tribolium castaneum*. Similarly, Regnault-Roger and Hamraoui (1995) reported that eugenol had weak insecticidal activity against *Acanthoscelides obtectus*. Contrarily, Zhang et al. (2016) stated that the LC_{50} value of eugenol against *D. melanogaster* was 0.003 μl/L. In addition, Xie et al. (2014b, 2015) also showed that eugenol was an effective fumigant against termites. These results suggested that the susceptibility of the same compounds to different insect species varied considerably. In this study, we also found that the fumigant activity of eugenol was higher than that of isoeugenol against *M. domestica*. Similar results were also obtained by Xie et al. (2014b, 2015) and Zhang et al. (2016). Contrarily, Huang et al. (2002) reported that isoeugenol was more effective than eugenol in insecticidal activity against *S. zeamais* and *T. castaneum*. This result indicated that the position of the double bond of the propenyl group is also very important in the insecticidal activity.

In this paper, two aldehydes (citral and cuminaldehyde) and one ketones ((+)-pulegone) were more effective than the tested alcohol compounds. Similar results were found in previous work, it has been found that some ketones and aldehydes were more effective fumigants than alcohols against housefly, stored product insects, Colorado potato beetle, *Culex pipiens*, *Reticulitermes chinensis*, and *D. melanogaster* (Rice and Coats 1994; Lee et al. 2003; Kordali et al. 2007; Abdelgaleil et al. 2009; Zahran and Abdelgaleil 2011; Xie et al. 2014b; Zhang et al. 2016). These observations raise the possibility that the presence of a carbonyl group augments the toxicity of compounds.

Although the compounds of hydrocarbon group exhibited weak insecticidal activity, ρ-cymene was more effective than other hydrocarbons that have double bonds. These results agree with those of Zhang et al. (2016), who also reported the antifungal activity of ρ-cymene was strong than other hydrocarbons. This result indicated that the addition of double bonds decreases potency, which may be related to lipophilicity.

In our study, the insecticidal activities of bornyl acetate, linalyl acetate, neryl acetate, citronellyl acetate, and geranyl acetate were generally more toxic than borneol, linalool, nerol, citronellol, and geraniol, respectively. Xie et al. (2014b) reported that bornyl acetate was more effective than borneol in insecticidal activity against *R. chinensis*. Similarly, it has been found that citronellyl acetate were more effective fumigants than citronellol against *D. melanogaster* (Zhang et al. 2016). This result indicates that the addition of an acetate group have increased the toxicity.

Conclusions

In this study, we investigated the insecticidal activities of 42 pure monoterpenes against housefly, *M. domestica*, which have not been reported previously. From the results of our present study, (+)-pulegone, cuminaldehyde, citral, and ρ-cymene showed good toxicity against the adult housefly. Thus, (+)-pulegone, cuminaldehyde, citral, and ρ-cymene could be used as potential natural insecticide for controlling housefly and could be useful in the search for new natural insecticidal against housefly.

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