ACTIVITY OF KAFFIR LIME (*CITRUS HYSTRIX*) ESSENTIAL OIL AGAINST BLOW FLIES AND HOUSE FLY

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Abstract. Blow flies and the house fly are not only pests but can be carriers of human pathogens. We aimed to determine the activity of the essential oil (EO) of the peel of Kaffir lime (*Citrus hystrix*) against 3 species of blow flies (*Chrysomya megacephala*, *Chrysomya rufifacies* and *Lucilia cuprina*) and the house fly (*Musca domestica*) in order to develop a plant derived method to control these pests. Larvicidal and adulticidal efficacy of *C. hystrix*'s EO were evaluated by dipping method and topical application, respectively. The EO studied gave lethal concentration 50 (LC_{50}) =38.93 g/l against *M. domestica*, a LC_{50}=61.00 g/l against *L. cuprina*, a LC_{50}=66.39 g/l against *C. rufifacies* and a LC_{50}=71.00 g/l against *C. megacephala*. Among female flies studied EO gave a lethal dose 50 (LD_{50}) =83.50 μg/fly against *M. domestica*, a LD_{50}=124.03 μg/fly against *C. megacephala*, a LD_{50}=210.46 μg/fly against *L. cuprina* and a LD_{50}=408.63 μg/fly against *C. rufifacies*. Scanning electron microscopy of the studied flies showed the studied EO resulted in a swollen, corroded integument with bleb formation. Light microscopy revealed a deformed midgut and hindgut and the fat cells having a vacuolated appearance. There was also a decrease in the number of nuclei in the fat cells and there were degeneration of the nuclei. Gas chromatography-mass spectrometry (GC-MS) evaluation of the studied EO revealed twenty-one compounds obtained by steam distillation. The major constituents were β-pinene (24.62%), sabinene (22.06%), limonene (19.29%), and citronellal (10.58%). Kaffir lime EO appears to be a potential candidate for further development as a plant derived method to control medically important fly species.

Keywords: *Citrus hystrix*, essential oil, blow flies, house fly, toxicity

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INTRODUCTION

Blow flies and house flies are not only pests but can spread human pathogens, such as bacteria, viruses, protozoa and helminth eggs (Greenberg, 1973; Sukon-

tason *et al*, 2007). Pathogenic bacteria found to be carried by these flies include *Salmonella* sp, *Shigella* sp and *Escherichia coli* O157:H7 (Chaiwong *et al*, 2014). The larvae of blow flies can also cause myiasis (Ferraz *et al*, 2010). In Thailand, 3 species of blow flies known to be medically important are *Chrysomya megacephala*, *Chrysomya rufifacies* and *Lucilia cuprina* and the house fly (*Musca domestica*) is also medically important (Ngoen-klan *et al*, 2011). These species can be found in and around human dwellings, especially during day-light hours. Since these flies live in or near human dwellings, they may be exposed to insecticides.

Conventional chemical pesticides are effective in fly control but continued, uncontrolled use of pesticides can lead to resistance (WHO, 1986; Kristensen *et al*, 2000). Chemical pesticides may have a negative effect on the environment, persist in the food chain and threaten nontarget organisms (Kristensen and Jespersen, 2003; Kumar *et al*, 2012b). This has resulted in a search for alternative botanical insecticides.

The essential oils (EO) of plants have been studied for their efficacy against flies (Kumar *et al*, 2012a; Morey and Khandagle, 2012). Kaffir lime (*Citrus hystrix* DC; family Rutaceae), known in Thailand as "*Ma-krud*", has been studied for its efficacy against mosquitoes (*Aedes aegypti*, *Anopheles dirus*, *Culex quinquefasciatus* (Tawatsin *et al*, 2001), *Anopheles minimus* (Nararak *et al*, 2017), cockroaches (*Periplaneta americana* (L.), *Blattella germanica* (L.), *Neostylopyga rhombifolia* (Stoll) (Thavara *et al*, 2007)), the larvae of *Ae. aegypti* (Sutthanont *et al*, 2010) and tobacco army worms (*Spodoptera litura*) (Loh *et al*, 2011). The efficacy of the EO of *C. hystrix* against medically important flies has not yet been studied in Thailand. Therefore, we aimed to: 1) determine the efficacy of the EO of *C. hystrix* against *C. megacephala*, *C. rufifacies*, *L. cuprina* and *M. domestica*; 2) determine the histopathological effects of this EO against the adults and larvae of the studied flies using a scanning electron microscope (SEM); and 3) determine the chemical composition of the studied EO.

Fig 1–Photograph of Kaffir lime (*Citrus hystrix*) fruit.

MATERIALS AND METHODS

Flies

The flies used in this study (*C. megacephala*, *C. rufifacies*, *L. cuprina* and *M. domestica*) were obtained from laboratory strains maintained for 9 years at ambient temperature in the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand. Fresh pork liver was provided for oviposition of the flies as described previously (Bunchu *et al*, 2008).

Essential oil preparation

C. hystrix (Fig 1) was purchased from a local market and identified by botanists in the Department of Biology, Faculty of Science, Chiang Mai University, Thailand. Voucher specimens were deposited at the

Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand. The peels were manually removed, shade dried at ambient temperature for 5-10 days and ground to coarse powder.

The ground *C. hystrix* powder was then distilled following the protocol of Champakaew *et al* (2015). About 250-300 g of ground *C. hystrix* powder was placed in an extraction column connected to a distillation flask containing ~1,600 ml of distilled water and 10-15 glass beads. The distilled water was heated to 100°C in an immersion heater to produce steam to pass through the plant materials. The distillate mixture of essential oil and water was collected and allowed to settle into 2 layers over 3-5 days with the EO on top of the water. The water was slowly released so only the EO remained. This was then dried with anhydrous sodium sulfate (Na_2SO_4) for 24 hours and then kept at 4°C until used.

Larvicidal toxicity assay

The lethal concentration of the EO of *C. hystrix* against the third instar larvae of *C. megacephala*, *C. rufifacies*, *L. cuprina* and *M. domestica* was determined following the method of Matsumura (1985). Five concentrations of the EO were prepared using 80% ethyl alcohol to prepare the dilutions. A negative control consisted of 80% ethyl alcohol without the EO. Thirty third instar larvae of each fly species tested (3 days after hatching from the same egg batch) were divided into 6 groups. Each group was placed in a fine net (each pore 334 μm×522 μm) and the net was then dipped into the respective studied solutions for 30 seconds. The dipped larvae by group were then placed in a tightly-sealed transparent plastic box (6.0×8.5×3.2 cm) containing pork liver (10 g cut into 2 small pieces). The mortality rate for each box was recorded at 24 hours after exposure; mortality was determined by touching each larva with a soft paint brush (No.0) and those not responding were considered dead. The experiment was conducted in triplicate for each group. The lethal concentrations (LC_{50}, LC_{90} and LC_{99}) were calculated with LdP line Software (Ehab Mostafa Bakr, Dokki, Cairo, Egypt).

Adulticidal bioassay

The lethal dose of the EO of *C. hystrix* against the studied adult flies followed the method of the WHO Expert Committee (1980). Approximately two hundred 3-5 day-old adult flies of each studied species were transferred from a rearing cage (30×30×30 cm) into a smaller cage (16×16×16 cm). The smaller cages were then placed in a large transparent plastic bag and CO_2 was then palced in the plastic bag to anesthetize the flies. After being anesthetized for 15 minutes, the small cage was removed from the plastic bag and all 200 flies were poured into a small plastic box and placed on an iceplate. Thirty flies were then randomly selected and placed on a Petri dish on an iceplate and then the studied solution was applied to each fly using an autopipette (Sartorius®, Goettingen, Germany). The flies were then placed back in the smaller cage again and given water and sugar *ad libitum* and kept at 28-32°C with 70-80% relative humidity. Each fly was then examined 24 hours after exposure to the studied EO to determine mortality. Each experiment was conducted in triplicate. The lethal dose (LD_{50}, LD_{90} and LD_{99}) was calculated using LdP line Software (Ehab Mostafa Bakr, Dokki, Cairo, Egypt). The EO preparations were made by diluting the EO with acetone to form 5 different concentrations.

Preparation of larvae for scanning electron microscopic examination

About 10-15 dead larvae of each studied fly species exposed to the highest dose of EO were randomly selected for scanning electron microscopy (SEM) analysis. Control larvae were also examined; they were killed by placing them in nearly boiling water for 10 minutes. The studied specimens were fixed with 2.5% glutaraldehyde and phosphate buffer solution at a pH of 7.4 at 4°C for 24 hours. They were rinsed twice with phosphate buffer solution 10 minutes apart. They were then treated with 1% osmium tetroxide at room temperature for 3-4 days post fixation and then rinsed twice with phosphate buffer solution. After that, they were dehydrated with alcohol using increasing concentrations: 50%, 70%, 80%, 90% and 95%. The specimens were placed in absolute alcohol for two 12-h periods, dried and then attached with double-stick tape to aluminum stubs, and coated with gold in a sputter-coating apparatus before being viewed with a JEOL JSM-5910 scanning electron microscope (Tokyo, Japan).

Histopathological evaluation

Five to ten larvae exposed to the highest dose of EO and control larvae were then prepared for histopathological evaluation by light microscopy. The specimens were fixed with 95% ethanol and 5% acetone and then dehydrated with increasing concentrations of alcohol: 70%, 80%, 95% and 100% (twice) and then mounted in paraffin blocks. Each specimen was sliced into 6 μm sections using a rotary microtome (Leitz 1512, Leica Microsystems, Wetzlar, Germany). The slices were then stained with hematoxylin and eosin and analyzed with light microscope (Olympus, Tokyo, Japan).

Chemical composition of the essential oil

The chemical composition of the EO of *C. hystrix* was determined by gas chromatography-mass spectrometry (GC/MS) using a Hewlette-Packard GC-MS system (Model 7890), following the method of Champakaew *et al* (2015). The GC/MS analysis was performed at the Science and Technology Service Center, Chiang Mai University (STSC-CMU), Thailand.

Statistical analysis

Statistical calculations were performed using LdP line Software (Ehab Mostafa Bakr, Dokki, Cairo, Egypt).

RESULTS

The lethal concentrations of the studied EO against the studied larvae are shown in Table 1. The highest LC_{50} was against *M. domestica* (LC_{50}=38.93 g/l), followed by *L. cuprina* (LC_{50}=61.00 g/l), *C. rufifacies* (LC_{50}=66.39 g/l) and *C. megacephala* (LC_{50}=71.00 g/l).

The lethal dosages of the studied EO against the studied adult flies are shown in Tables 2 and 3. The LD_{50} was obtained in female fly against *M. domestica* (LD_{50}=83.50 μg/fly), *C. megacephala* (LD_{50}=124.03 μg/fly), *L. cuprina* (LD_{50}=210.46 μg/fly) and *C. rufifacies* (LD_{50}=408.63 μg/fly). Similar results were observed against males of *M. domestica* (LD_{50}=81.06 μg/fly), *C. megacephala* (LD_{50}=117.98 μg/fly), *L. cuprina* (LD_{50}=143.04 μg/fly) and *C. rufifacies* (LD_{50}=386.90 μg/fly).

On SEM the control larvae of all the species examined had normal body architecture, a smooth outer integument contour (Fig 2A, C, E and G). The EO-treated larvae species had disfigured body architecture with a swollen integument in *M. domestica*, *C. megacephala* and *L. cuprina* (Fig 2B, D and F). There was bleb formation along the integument. A thick residuum was observed coating the

Table 1
Larvicidal activity of the essential oil of *C. hystrix* against the third instar larvae of the studied flies.

Concentrations (g/l)	% mortality (mean±SE)	*n*	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	LC_{99} (LCL-UCL)	slope	χ^2	df
C. megacephala								
53.75	14.44±10.72	90	71	93.66	117.38	10.66	8.35	4
64.50	26.67±6.67		(64.68-76.95)	(89.10-116.14)	(111.40-168.68)			
75.68	56.67±23.09							
80.84	70.00±26.03							
86.00	88.89±10.18							
C. rufifacies								
45.26	26.67±3.30	90	66.39	139.77	256.43	3.96	4.68	4
64.73	41.11±3.85		(61.03-71.50)	(122.91-168.41)	(204.86-359.40)			
74.79	60.00±8.82							
95.55	80.00±10.00							
151.76	90.00±10.00							
L. cuprina								
45.26	28.89±21.69	90	61	82.44	105.37	9.80	6.11	4
64.73	60.00±8.82		(58.36-63.22)	(78.83-87.62)	(97.34-118.29)			
74.79	80.00±12.02							
95.55	94.44±1.92							
151.76	96.67±3.33							
M. domestica								
26.60	1.11±1.92	90	38.93	49.29	59.75	12.50	3.54	4
31.19	15.56±18.95		(37.71-40.60)	(46.12-54.66)	(54.02-70.09)			
35.84	26.67±17.32							
40.52	61.11±15.75							
45.21	96.67±5.77							

LC_{50} lethal concentration that kills 50% of the exposed larvae; LC_{90} lethal concentration that kills 90% of the exposed larvae; LC_{99} lethal concentration that kills 99% of the exposed larvae; UCL: upper confidence limit; LCL: lower confidence limit; χ^2 = chi-square; df = degrees of freedom; *n* = number of flies tested. Units are in g/l.

Table 2
Adulticidal activity of the essential oil of *C. hystrix* against female flies.

Concentrations (μg/fly)	% mortality (mean±SE)	*n*	LD_{50} (LCL-UCL)	LD_{90} (LCL-UCL)	LD_{99} (LCL-UCL)	slope	χ^2	df
C. megacephala								
68.80	10.00±6.61	90	124.03	203.26	304.04	5.97	9.75	4
94.60	40.83±11.81		(108.12-144.03)	(187.07-289.48)	(279.11-535.89)			
120.40	42.50±5.00							
146.20	61.67±7.64							
172.00	87.50±6.61							
C. rufifacies								
86.00	2.22±1.92	90	408.63	673.25	1,011.43	5.91	4.45	4
172.00	2.22±17.32		(384.80-434.38)	(602.59-798.96)	(842.29-1,353.94)			
344.00	32.22±19.25							
430.00	47.78±18.95							
516.00	78.89±5.09							
L. cuprina								
43.00	3.33±3.33	90	210.46	381.12	618.42	4.97	0.60	4
86.00	5.56±1.92		(194.27-226.78)	(348.10-425.06)	(539.96-735.68)			
172.00	31.11±1.92							
344.00	85.56±15.03							
430.00	96.67±3.33							
M. domestica								
68.80	41.11±16.78	90	83.5	173.51	314.97	4.03	3.42	4
94.60	54.44±1.92		(74.57-90.79)	(155.15-204.87)	(254.64-440.28)			
120.40	70.00±12.02							
146.20	83.33±14.53							
172.00	93.33±8.82							

LC_{50} lethal concentration that kills 50% of the exposed larvae; LC_{90} lethal concentration that kills 90% of the exposed larvae; LC_{99} lethal concentration that kills 99% of the exposed larvae; UCL: upper confidence limit; LCL: lower confidence limit; χ^2 = chi-square; df = degrees of freedom; *n* = number of flies tested. Units are μg/fly.

Table 3
Adulticidal activity of the essential oil of *C. hystrix* against male flies.

Concentrations (μg/fly)	% mortality (mean±SE)	*n*	LD_{50} (LCL-UCL)	LD_{90} (LCL-UCL)	LD_{99} (LCL-UCL)	slope	χ^2	df
C. megacephala								
68.80	10.83±2.89	90	117.98	210.10	336.28	5.11	3.65	4
94.60	35.83±12.83		(112.23-124.04)	(191.12-238.74)	(287.46-417.73)			
120.40	47.50±17.32							
146.20	65.00±16.39							
172.00	83.33±11.27							
C. rufifacies								
86.00	2.22±3.85	90	386.90	626.73	928.62	6.12	1.45	4
172.00	5.56±3.85		(364.05-409.80)	(568.66-723.97)	(789.61-1,192.65)			
344.00	33.33±6.67							
430.00	85.56±6.94							
516.00	85.56±6.94							
L. cuprina								
43.00	2.22±1.92	90	143.04	259.05	420.38	4.97	9.57	4
86.00	12.22±5.09		(114.62-179.44)	(235.19-461.39)	(390.89-1,077.01)			
129.00	34.44±1.92							
172.00	64.44±22.19							
215.00	86.67±5.77							
M. domestica								
68.80	41.11±5.09	90	81.06	172.99	320.92	3.89	5.87	4
94.60	61.11±12.62		(71.58-88.65)	(154.16-205.78)	(256.85-458.53)			
120.40	71.11±15.03							
146.20	78.89±10.18							
172.00	95.56±5.09							

LC_{50} lethal concentration that kills 50% of the exposed larvae; LC_{90} lethal concentration that kills 90% of the exposed larvae; LC_{99} lethal concentration that kills 99% of the exposed larvae; UCL: upper confidence limit; LCL: lower confidence limit; χ^2 = chi-square; df = degrees of freedom; *n* = number of flies tested. Units are μg/fly.

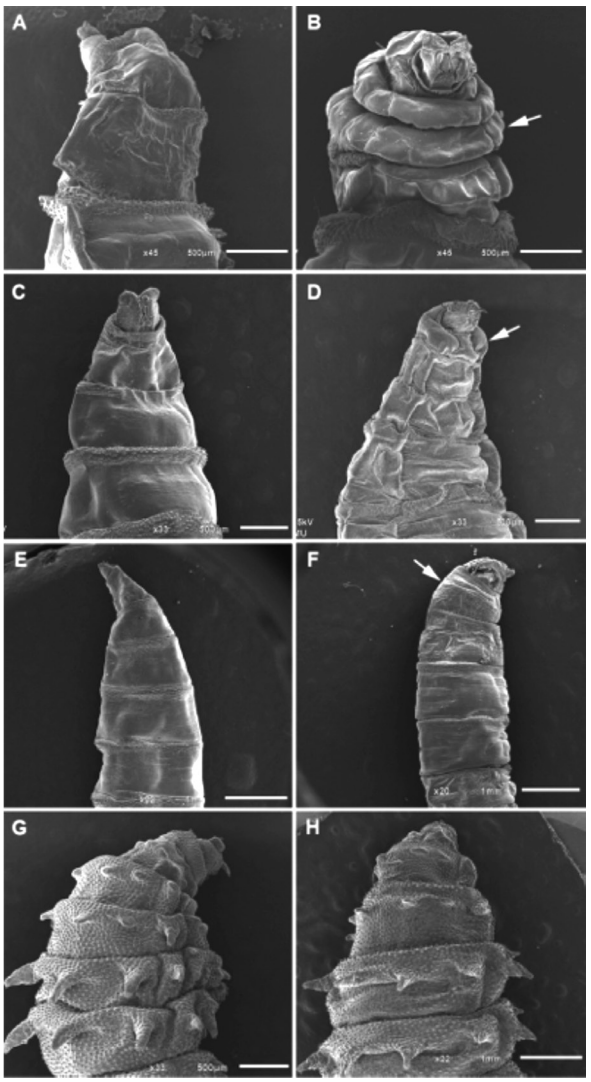

Fig 2–Scanning electron micrographs of anterior end of third instar larvae of flies treated with essential oil of *C. hystrix*. (A) *Chrysomya megacephala*, control. (B) *Chrysomya megacephala*, treated, showing swollen integument (arrow). (C) *Lucilia cuprina*, control. (D) *Lucilia cuprina*, treated, showing swollen integument (arrow). (E) *Musca domestica*, control. (F) *Musca domestica*, treated, showing swollen integument (arrow). (G) *Chrysomya rufifacies*, control. (H) *Chrysomya rufifacies*, treated, showing no difference from control.

posterior spiracle. No pathological changes were seen in the *C. rufifacies* control or treatment groups (Fig 2G and H).

Light microscopy of the larvae in the control group revealed a normal structure (Figs 3A-C, 4A-C). Treated larvae had damaged contours with re-organized cellular components (Fig 3D and 4D). The columnar epithelial cells of the midgut had more vacuolization in the cytoplasm (Fig 3E and 4E) and the membranous sheath had detached from the basement membrane. The fat cells were markedly deformed, less vacuolated and had degenerated nuclei and cytoplasms (Fig 3F and 4F).

The EO of *C. hystrix* was a pale yellow clear liquid with a strong lemon/lime odor. Gas chromatography revealed 21 components (Table 4); the main being β-pinene (24.62%), sabinene (22.06%), limonene (19.29%) and citronellal (10.58%).

DISCUSSION

The use of botanical insecticides has received much attention because it is thought by some to be more biodegradable, more easily available, less toxic to higher animals and more eco-friendly to the environment (Bowers, 1992). Few studies have evaluated the efficacy of the studied EO against nuisance flies. The EO of tea tree (*Melaleuca alternifolia*) was found to be larvicidal against the blow fly *L. cuprina* (Callander and James, 2012). The EO of *Artemisia* spp was found to be toxic to the blow fly *Calliphora vomitoria* on contact and with fumigation (Bedini *et al*, 2017). Our results show the EO of *C. hystrix* has some activity against nuisance flies.

Kaffir lime oil has been used as an insecticide and a repellent of mosquito

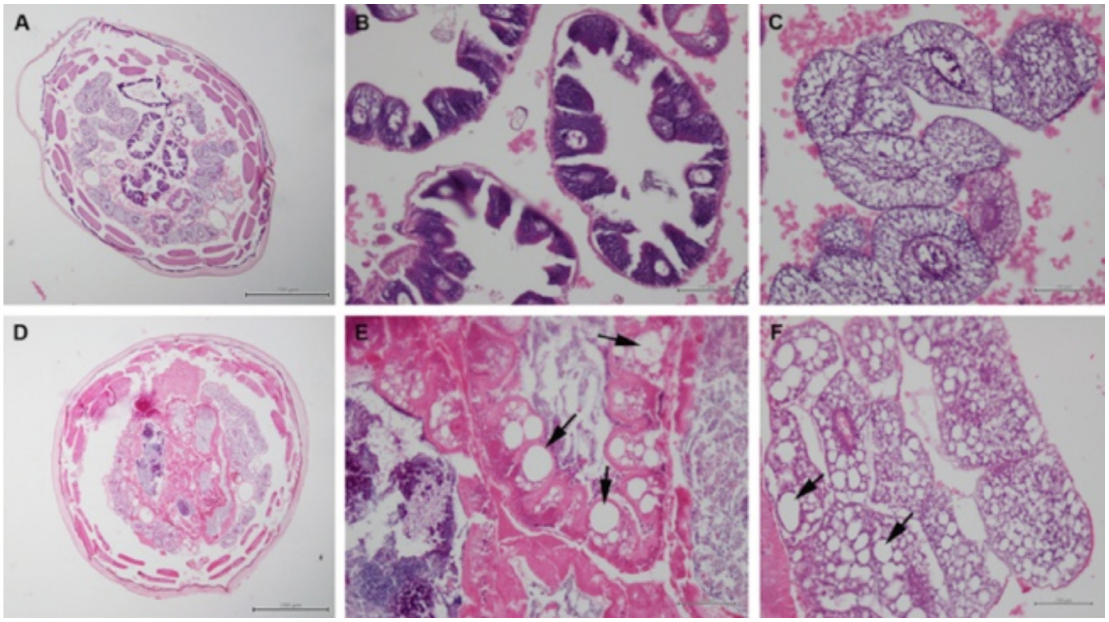

Fig 3–Histological sections of third instar larvae of *M. domestica* stained with hematoxylin and eosin. (A-C) Control treatment with no exposure to essential oil of *C. hystrix*. (A) Control larva showing normal contour of structure, dense muscles and fat cells are seen. (B) Normal midgut showing well-arranged single layered epithelium of columnar cells. (C) Normal fat cells a normal configuration and cytoplasm engorged with inclusions. (D-F) Exposure of the EO of *C. hystrix*. (D) Treated larva showing decrease muscle and fat cells. (E) Deformation and vacuolation of midgut cells (arrows). (F) Vacuolation of fat cells (arrows).

vectors, cockroaches and tobacco army worms (Tawatsin *et al*, 2001; Thavara *et al*, 2007; Sutthanont *et al*, 2010; Nararak *et al*, 2017). In our study, based on the results of the LC_{50} calculations the EO of *C. hystrix* was most effective against *M. domestica*. The LC_{50} values varied by species (*M. domestica*: LC_{50} = 38.93 g/l); *C. megacephala*: LC_{50} = 71.0 g/l).

Similar results were observed in previous study using neem extract on the larvae of *M. domestica* and *C. megacephala*, which showed the former species more susceptible than the latter (Siriwattanarungsee *et al*, 2008). This may be explained by size, since the third instar of *M. domestica* are smaller than the sizes of the other three studied species. Of the three blow fly species studied, *L. cuprina* larvae are smaller than *C. megacephala* and *C. rufifacies*. The integument is important for the protection of insects. The larvae of *M. domestica* have thinner integument than *C. megacephala* and *C. rufifacies*. The smooth integument of *M. domestica*, *L. cuprina* and *C. megacephala* could make them more susceptible to EO than the net-like patch integument and hairy tubercles of *C. rufifacies* (Sukontason *et al*, 2003). Other factors may also influence susceptibility to the studied EO; further studies are needed to determine these factors. Both SEM and light microscopy revealed damage to the studied fly larvae caused by the EO, similar to a previous study (Sukontason *et al*, 2004). These findings raise questions

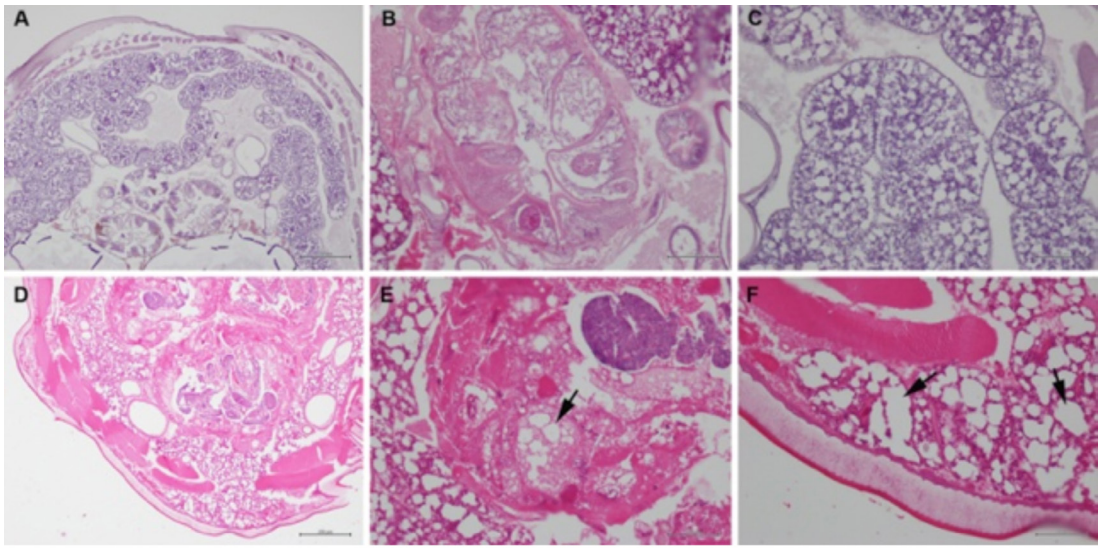

Fig 4–Histological sections of third instar larvae of *L. cuprina* stained with hematoxylin and eosin. (A-C) Control treatment with no exposure to the essential oil of *C. hystrix*. (A) Control larva showing normal contour of structure, dense fat cells are seen. (B) Normal midgut showing well-arranged single layered epithelium of columnar cells. (C) Normal fat cells showing normal configuration and cytoplasm engorged with inclusions. (D-F) Exposed to EO *C. hystrix*. (D) Treated larva. (E) Disorganization and vacuolation of midgut cells (arrow). (F) Vacuolation of fat cells (arrows).

about the effect of the EO on the reproductive organs of the fly species.

Regarding the adulticidal assay, the EO had the greastest activity against both male and female *M. domestica*, followed by *C. megacephala*, *L. cuprina* and *C. rufifacies*. A previous report using the commercial eucalyptol (Sigma-Aldrich®, Switzerland) on *M. domestica* and *C. megacephala* also showed similar activity (Sukontason *et al*, 2004); however, the LD_{50} in both sexes of these species was found to be higher in eucalyptol than in the EO in this study. A possible explaination for this may be due to the difference in active compounds in each plant. Furthermore, the commonly used insecticides, permetrin and deltametrin, have been recommended by the WHO for house fly control, with the LD_{50} being 0.02 and 0.001 µg/fly, respectively (WHO, 1986). A lower LD_{50} of insecticide might involve defined active compounds, whereas the EO of *C. hystrix* contains several phytochemicals (active and/or inactive compounds), which lead to the use of high dosage (Mansour *et al*, 2012). Although chemical insecticides can reduce effectively fly populations, their negative effects can result in residuals in the environment, non-target organisms, and their long-term use causing resistance in target organisms (WHO, 1980).

There is little data regarding the chemical composition of the EO of *C. hystrix* (Manosroi *et al*, 1999; Chanthaphon *et al*, 2008; Kerdchoechuen *et al*, 2010; Waikedrea *et al*, 2010). In our study, the majority of the compounds identified belonged to the monoterpenes. Chromatography of our studied EO showed β-pinene

Table 4
Chemical composition of the compounds identified in the essential oil of *C. hystrix* using gas chromatography-mass spectrometry analysis.

Peak	Chemical constituent	Retention time (min)	Area (%)
1	α-thujene	6.28	0.35
2	α-pinene	6.49	4.30
3	sabinene	7.53	22.06
4	β-pinene	7.71	24.62
5	β-myrcene	7.85	1.92
6	p-cymene	8.84	0.88
7	limonene	9.03	19.29
8	γ-terpinene	9.75	0.40
9	unidentified	10.12	0.74
10	linalool	10.91	0.97
11	citronellal	12.45	10.58
12	terpinen-4-ol	13.27	2.31
13	α-terpineol	13.67	1.55
14	unidentified	14.13	0.65
15	β-citronellol	14.49	1.78
16	propanal, 2-methyl-3-phenyl	14.99	1.44
17	2,6 octadiene, 2,6-dimethyl	17.80	1.01
18	α-cubebene	18.57	2.05
19	β-cubebene	18.88	1.14
20	caryophyllene	19.73	0.76
21	δ-cadiene	22.13	1.20
Total			100.00

(24.62%), sabinene (22.06%), limonene (19.29%) and citronellal (10.58%). A similar study from Thailand of the EO of kaffir lime found β-pinene (30.6%), limonene (29.2%), sabinene (22.6%) and citronellal (4.2%) (Manosroi *et al*, 1999). Another study (Chanthaphon *et al*, 2008) found β-pinene (30.48%), limonene (8.13%), sabinene (22.75%) and citronellal (15.67%) using hydrodistillation. Kerdchoechuen *et al* (2010) reported finding limonene (38.6%) and β-pinene (30.5%). Waikedrea *et al* (2010) found terpinen-4-ol (13.0%) and β-pinene (10.9%) using hydrodistillation. Such variations in chemical composition may be due to several factors: the plant part (fruit *vs* leaf), the ripeness of the fruit, the location of the plantation, storage conditions and extraction procedures (Lota *et al*, 2000). Our finding of β-pinene as the major component of *C. hystrix* is consistent with studies of a *Citrus* species, *Citrus limon* (L.) Burm. f. (Simas *et al*, 2017). It was also reported as the second most common component of the EO of the fruit of *Citrus limon* (Ghoorchibeigi *et al*, 2017). β-pinene, limonene and sabinene have also been isolated from the EO of other plants, such as *Stachys officinalis* (L.) Trevisan subsp. *officinalis* (Lamiaceae) (Giuliani *et al*, 2017).

The second most common component found in our study, limonene, has been found in *Citrus limonia* Osbeck (up to

65.7%), *C. latifolia* Tanaka ex Q. Jimenez (35.4%) and *C. aurantifolia* (Christm.) Swingle (31.1%) (Simas *et al*, 2017). It has also been found in *Citrus aurantium* L.var *amara* (87.02 %) (Trabelsi *et al*, 2016) and *Citrus limon* (61.4%) (Ghoorchibeigi *et al*, 2017).

In conclusion, the EO of *C. hystrix* damaged the integument of the larvae of the studied fly species, causing mortality. Further studies are needed to determine the efficacy of this EO in actual use. Our results reveal a potential control method for the studied fly species.

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