EFFICACY OF HERBAL ESSENTIAL OILS AS INSECTICIDE AGAINST *AEDES AEGYPTI* (LINN.), *CULEX QUINQUEFASCIATUS* (SAY) AND *ANOPHELES DIRUS* (PEYTON AND HARRISON)

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Abstract. The essential oils of *Cananga odorata* (ylang ylang), *Citrus sinensis* (orange), *Cymbopogon citratus* (lemongrass), *Cymbopogon nardus* (citronella grass), *Eucalyptus citriodora* (eucalyptus), *Ocimum basilicum* (sweet basil) and *Syzygium aromaticum* (clove), were tested for their insecticide activity against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles dirus* using the WHO standard susceptibility test. These were applied in soybean oil at dose of 1%, 5% and 10% (w/v). *C. citratus* had the KT_{50} values against the three mosquito species tested but the knockdown rates (at 10, 30 and 60 minutes) were lower than some essential oils. *C. citratus* oil had high insecticidal activity against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. dirus*, with LC_{50} values of <0.1, 2.22 and <0.1%, respectively. Ten percent *C. citratus* gave the highest mortality rates (100%) 24 hours after application. This study demonstrates the potential for the essential oil of *C. citratus* to be used as an insecticide against 3 species of mosquitoes.

Keywords: essential oil, insecticidal activity, *Aedes aepypti*, *Culex quinquefasciatus*, *Anopheles dirus*

INTRODUCTION

Aedes aegypti (L.), *Culex quinquefasciatus* (Say) and *Anopheles dirus* (Peyton and Harrison) mosquitoes are widely distributed in rural and urban areas of Thailand. *Ae. aegypti* is a major vector of dengue fever (DF) and dengue hemorrhagic fever (DHF). *Cx. quinquefasciatus* is a vector for Japanese encephalitis (JE) and causes annoyance and dermatitis. *An. dirus* is an important malaria vector along the border of Thailand. There is currently only a vaccine against JE, but not malaria or dengue infection; therefore, only vector control measures are available (Shultz *et al*, 2008). Insecticides are needed, especially during epidemics of disease. Insecticides remain the mainstay of mosquito vector control programs. Synthetic pyrethroids and DDT were previously effective but resistance to these chemicals has occurred (N'Guessan *et al*, 2007) among *Ae. aegypti* (Paeporn *et al*, 2004; Yaicharoen *et al*, 2005), *Cx. quinquefasciatus* (Sathantriphop *et al*, 2006) and *An. dirus* (Van Bortel *et al*, 2008).

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Synthetic insecticides may have adverse environmental effects, high cost and poor community acceptance.

Essential oils have received attention as potentially controlling vectors of mosquito-borne disease (Sutthanont *et al*, 2010). Natural products used as insecticides may have less of an environmental impact due to shorter latency, possibly resulting in reduced resistance (Hardin and Jackson, 2009). Sukumar *et al* (1991) evaluated 344 different plant species to control mosquitoes instead of synthetic chemical insecticides. Some essential oil derived compounds may be used on humans similar to some conventional insecticides, are selective and have fewer harmful effects than some conventional insecticides (Mumcuoglu *et al*, 2002).

The aim of this study was to determine the mosquitocidal activities of seven herbal essential oils: *Cananga odorata*, *Citrus sinensis*, *Cymbopogon citratus*, *C. nardus*, *Eucalyptus citriodora*, *Ocimum basilicum* and *Syzygium aromaticum*, against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. dirus* using a standard WHO susceptibility test at varying concentrations under laboratory conditions (WHO, 1998).

MATERIALS AND METHODS

Mosquitoes

Ae. aegypti, *Cx. quinquefasciatus* and *An. dirus* mosquitoes were used in this study. *Ae. aegypti* and *An. dirus* eggs were obtained from the Armed Forces Research Institute of Medical Sciences (AFRIMS). *Cx. quinquefasciatus* eggs were obtained from the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University. They were maintained at the laboratory of Entomology and Environment, Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut Institute of Technology Lat Krabang (KMITL), Bangkok. Mosquitoes were reared at 30-35^{o}C and 70-80% relative humidity. Larvae were reared on a diet of floating fish food. The adults were maintained in screened cages and fed 5% glucose solution in water soaked on cotton pads. One to two day old emerging female mosquitoes were randomly selected for adult bioassays. Prior to testing, they were starved by providing them with only water for 12 hours.

Plant materials

Cananga odorata Lamk. (ylang ylang flowers), *Citrus sinensis* L. Osbeck (orange fruits), *Cymbopogon citratus* DC. Stapf (lemongrass leaves and stems), *Cymbopogon nardus* L (citronella grass leaves), *Eucalyptus citriodora* Hook (eucalyptus leaves), *Ocimum basilicum* L. (sweet basil leaves) and *Syzygium aromaticum* L. (clove flowers) were collected and the essential oils were extracted by steam distillation, and prepared as 1%, 5% and 10% solutions in soybean oil. All formulations were kept at room temperature before testing.

WHO susceptibility test

Susceptibility testing was carried out using the standard WHO protocol (1998) using diagnostic kits and impregnated paper. Each tube was marked with a red spot or a green spot, indicating its use for holding insecticide paper or for holding mosquitoes 1 hour after exposure. Twenty-five mosquitoes were collected from a cage with an aspirator and blown into green spot tubes lined with clean white paper, with a movable slide attached. Filter paper (Whatman® No.1) 12cm x 15cm was cut for the bioassay. The filter paper was treated with essential oils and placed into red spot tubes. These were screwed onto the opposite side of the movable slide; the slide was then opened and the

Table 1

KT_{50} and LC_{50} values caused by seven essential oils against *Ae. aegypti* adult mosquitoes.

Herbal essential oils	1%			5%			10%			LC_{50} (%)
	KT_{50} (min)	% Mortality	Susceptibility	KT_{50} (min)	% Mortality	Susceptibility	KT_{50} (min)	% Mortality	Susceptibility	
Cananga odorata oil	151.94	8.8^{bc}	R	168.06	14.4^{d}	R	21.33	54.4^{d}	R	9.77
Citrus sinensis oil	339.86	16.8^{bc}	R	100.7	55.2^{bc}	R	76.69	79.2^{bc}	R	5.26
Cymbopogon citratus oil	27.31	74.4^{a}	R	11.06	86.4^{a}	RS	<1.00	100.0^{a}	S	<0.10
Cymbopogon nardus oil	53.28	60.0^{a}	R	15.85	69.6^{ab}	R	1.22	97.6^{a}	RS	0.26
Eucalyptus citriodora oil	108.48	59.2^{a}	R	41.92	76.8^{ab}	R	20.42	95.2^{ab}	R	<0.10
Ocimum basilicum oil	307.53	23.2^{b}	R	208.86	41.6^{c}	R	62.18	70.4^{c}	R	6.31
Syzygium aromaticum oil	37.79	58.4^{a}	R	16.05	68.8^{ab}	R	3.44	93.6^{ab}	R	0.12
Negative control (Soybean oil)		0.0^{c}			0.0^{d}			0.0^{e}		
CV (%)		41.7			32.4			16.7		

KT_{50}, 50% knockdown time; LC_{50}, 50% lethal concentration.

Mean % mortality followed by the same letter in the same column are not significantly different (one-way ANOVA and Duncan's multiple range test).

S, Susceptible is defined as 98-100% mortality; RS, Resistance suspected is defined as 80-97% mortality; R, Reisitance is defined as <80% mortality.

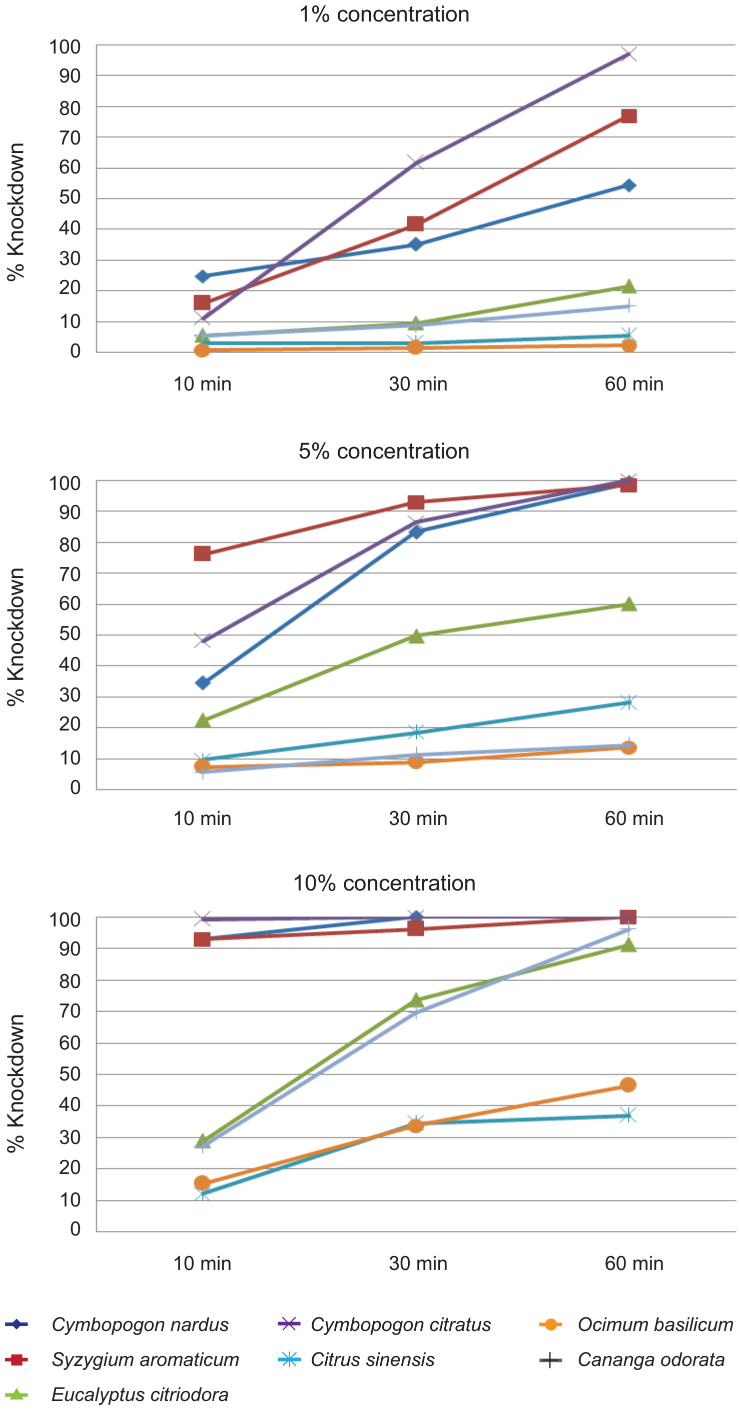

Fig 1–Percent knockdown of female *Ae. aegypti* mosquitoes by 1, 5 and 10% concentrations of seven essential oils after 10, 30 and 60 minutes of exposure.

mosquitoes gently blown into the red spot tube after which the slide was closed again. Twenty-five female mosquitoes were exposed to the impregnated paper for 1 hour in each tube while the mosquitoes used as controls were exposed to paper without insecticide and then gently blown back into the green spot tube. Each test was performed in five replicates with simultaneous control sets; the negative controls were impregnated with soybean oil only. Knockdown rates were recorded at 10, 30 and 60 minutes during the 1 hour exposure. At the end of the exposure period mosquitoes were transferred to recovery tubes and provided with 5% glucose solution. Mortality was observed after 24 hours.

Statistical analysis

KT_{50} and LC_{50} values were calculated using probit analysis. The mortality data was analyzed with the Duncan's multiple range test using SPSS for Windows (version 16.0).

The susceptibility results were determined for each insecticide using WHO criteria (WHO, 1998): 98-100% mortality indicated susceptibility, 80-97% mortality suggested possible resistance needing confirmation and mortality <80% suggested resistance. Mor-

Table 2
KT_{50} and LC_{50} values for *Cx. quinquefasciatus* adult mosquitoes to seven herbal essential oils.

Herbal essential oils	1%			5%			10%			LC_{50} (%)
	KT_{50} (min)	% Mortality	Susceptibility	KT_{50} (min)	% Mortality	Susceptibility	KT_{50} (min)	% Mortality	Susceptibility	
Cananga odorata oil	*	5.6^{c}	R	100.87	20.8^{bcd}	R	1.7	60.0^{b}	R	8.82
Citrus sinensis oil	83.47	2.4^{c}	R	170.87	22.4^{bcd}	R	72.28	16.8^{d}	R	20.8
Cymbopogon citratus oil	20.08	38.4^{a}	R	7.75	75.2^{a}	R	<1.00	100.0^{a}	S	2.22
Cymbopogon nardus oil	29.16	28.0^{ab}	R	11.17	43.2^{b}	R	<1.00	100.0^{a}	S	4.12
Eucalyptus citriodora oil	100.2	8.0^{c}	R	41.11	13.6^{cd}	R	5.77	40.0^{c}	R	12.16
Ocimum basilicum oil	48.19	12.8^{bc}	R	18.22	18.4^{cd}	R	1.66	47.2^{bc}	R	10.98
Syzygium aromaticum oil	21.51	16.0^{bc}	R	16.05	35.2^{bc}	R	<1.00	100.0^{a}	S	4.99
Negative control (Soybean oil)		0.0^{c}	R		0.0^{d}	R		0.0^{d}	R	
CV (%)		97.9			57.5			24.2		

KT_{50}, 50% knockdown time; LC_{50}, 50% lethal concentration.
Mean % mortality followed by the same letter in the same column are not significantly different (one-way ANOVA and Duncan's multiple range test).
S, Susceptible is defined as 98-100% mortality; RS, Resistance suspected is defined as 80-97% mortality; R, Resistance is defined as <80% mortality.
*not computed by Probit analysis

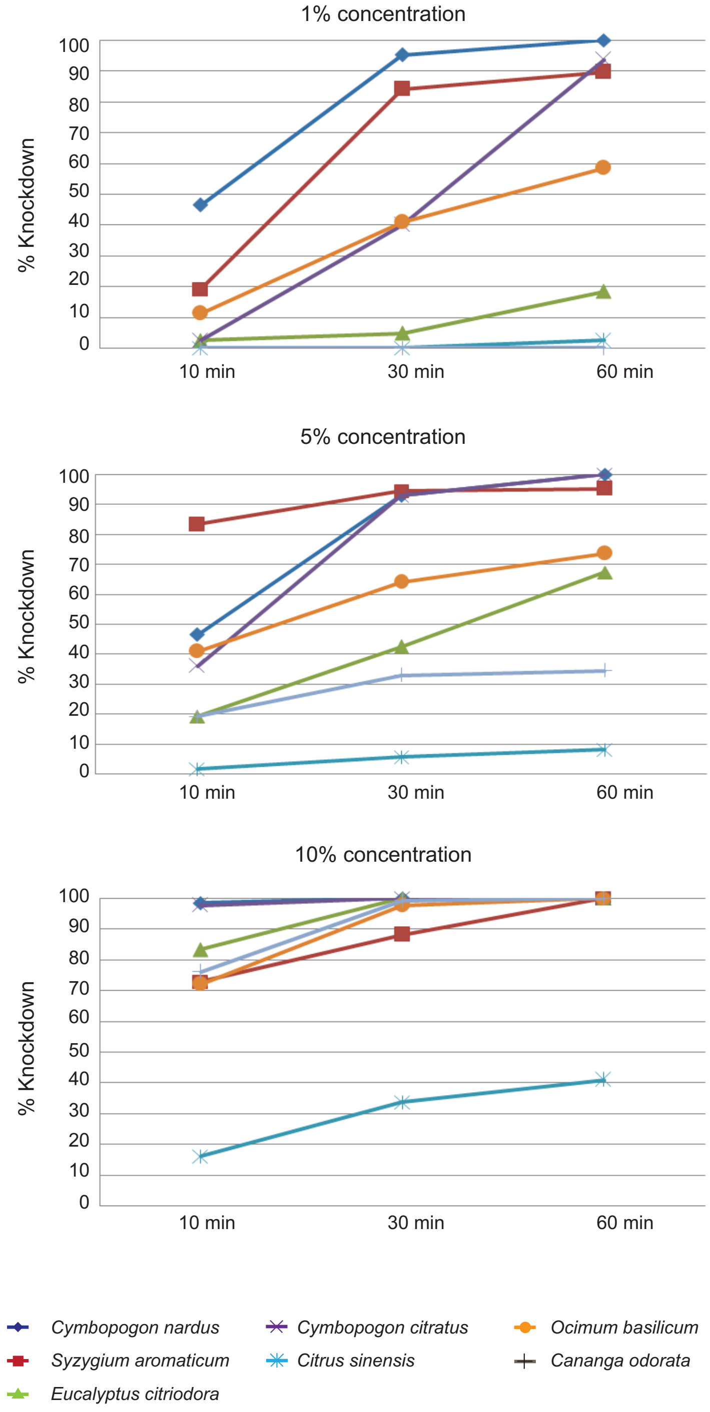

Fig 2–Percent knockdown of female *Cx. quinquefasciatus* mosquitoes by 1, 5 and 10% concentrations of seven essential oils after exposure at 10, 30 and 60 minutes.

tality on the WHO resistance tests was corrected for control mortality using Abbott's formula (Abbott, 1987) when control mortality was 5-20%. Experimental tests with >20% control mortality were discarded and repeated.

RESULTS

One kg of each plant was used for this study to obtain the essential oils as follows: 2.5% (w/v) *Ca. odorata*, 2.0% (w/v) *Ci. sinensis*, 2.6% (w/v) *C. citratus*, 2.2% (w/v) *C. nardus*, 2.4% (w/v) *E. citriodora*, 1.3% (w/v) *O. basilicum* and 3.0% (w/v) *S. aromaticum*. Three essential oil-soybean oil concentrations were used for this study: 1%, 5% and 10%. Mortality with the seven tested essential oils was determined using essential oil impregnated papers using WHO test kits against *Ae. aegypti* as shown in Table 1. The highest KT_{50} values for *C. citratus* (at 1, 5 and 10% concentration) were 27.31 minutes, 17.36 minutes and <1 minute, respectively. Fig 1 shows the knockdown rates at 10, 30 and 60 minutes. Ten percent *C. citratus* gave better knockdown rates from 10 minutes to 60 minutes than the other oils. Ten percent *C. citratus* and 10% *C. nardus* gave mortality rates of 100% and 97.6%, respectively. There were significant overall among the efficacy of the essential oils against *Ae. aegypti* (p<0.05). The LC_{50} values of the seven essential oils ranged from

Table 3

KT_{50}, LC_{50} values, mortality rates and susceptibility of *An. dirus* adults to seven herbal essential oils.

Herbal essential oils	1%			5%			10%			LC_{50} (%)
	KT_{50} (min)	% Mortality	Susceptibility status	KT_{50} (min)	% Mortality	Susceptibility status	KT_{50} (min)	% Mortality	Susceptibility status	
Cananga odorata oil	127.82	24.0^{c}	R	36.49	40.8^{b}	R	2.44	89.6^{ab}	RS	4.99
Citrus sinensis oil	196.89	23.2^{c}	R	110.19	40.0^{b}	R	14.92	83.2^{b}	RS	5.45
Cymbopogon citratus oil	2.39	92.8^{a}	RS	<1.00	97.6^{a}	RS	<1.00	100.0^{a}	S	<0.10
Cymbopogon nardus oil	10.9	76.8^{ab}	R	<1.00	93.6^{a}	RS	<1.00	100.0^{a}	S	<0.10
Eucalyptus citriodora oil	54.58	59.2^{b}	R	<1.00	95.2^{a}	RS	<1.00	91.2^{ab}	RS	<0.10
Ocimum basilicum oil	61.34	60.8^{b}	R	17.15	53.6^{b}	R	<1.00	76.8^{b}	R	<0.10
Syzygium aromaticum oil	9.08	51.2^{b}	R	<1.00	54.4^{b}	R	<1.00	100.0^{a}	S	2.11
Negative control (Soybean oil)		0.0^{c}			0.0^{c}			0.0^{c}		
CV (%)		41. 7			26.7			14.0		

KT_{50}, 50% knockdown time; LC_{50}, 50% lethal concentration.

Mean % mortality followed by the same letter in the same column is not significantly different (one-way ANOVA and Duncan's multiple range test).

S, Susceptible is defined as 98-100% mortality; RS, Resistance suspected is defined as 80-97% mortality; R, Reisitance is defined as <80% mortality.

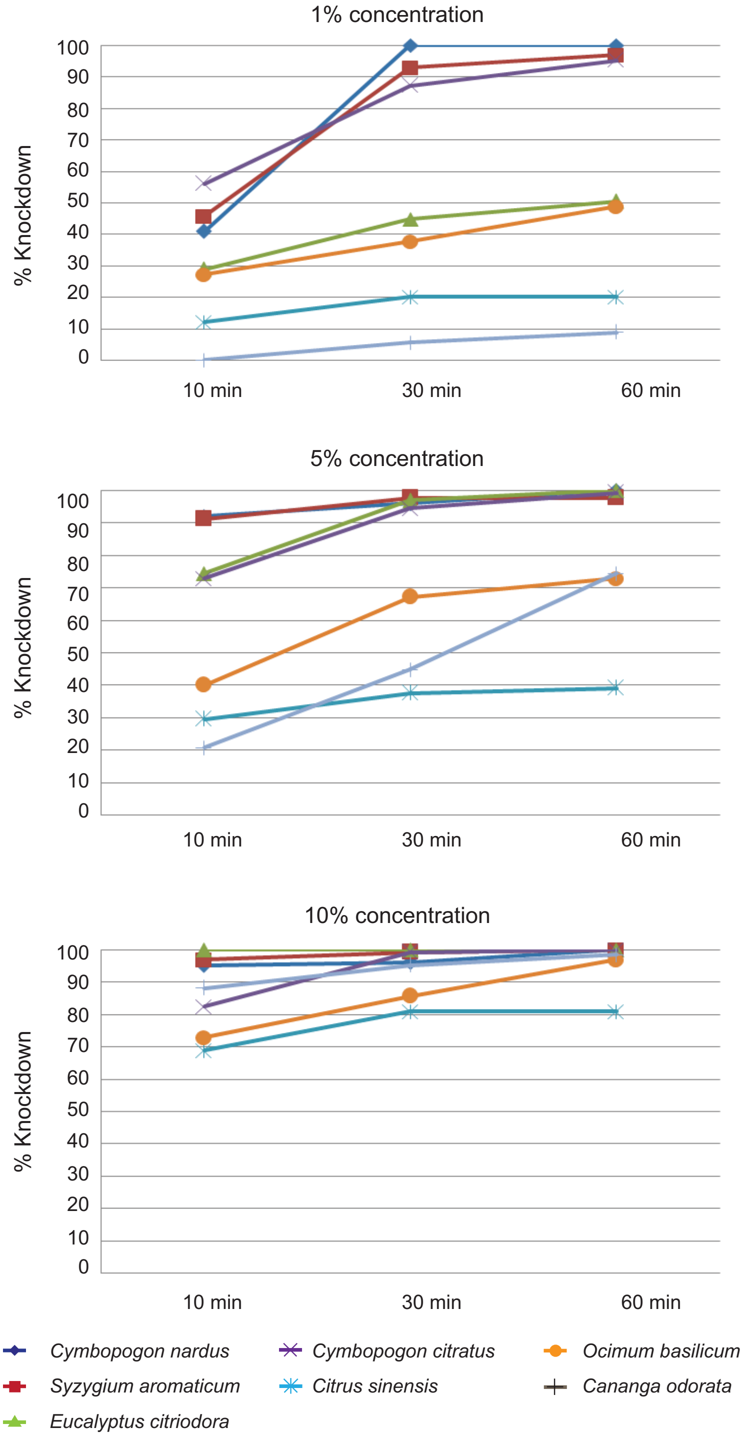

Fig 3–Percent knockdown of female *An. dirus* mosquitoes by 1, 5 and 10% concentrations of seven essential oils at 10, 30 and 60 minutes of exposure.

<0.1% to 9.77% 24 hour after exposure.

One percent and 5% *C. citratus* gave KT_{50} values of 20.08 and 7.75, respectively, against *Cx. quinquefasciatus* (Table 2). At 1% and 5% *C. citratus* gave knockdown rates lower than *C. nardus* and *S. aromaticum* (Fig 2). *Cx. quinquefasciatus* was resistant to all seven essential oils at oil concentrations of 1, 5 and at 10% it was resistant to all essential oils, except for *C. citratus*, *C. nardus* and *S. aromaticum*. The susceptibilities of adult *Cx. quinquefasciatus* mosquitoes to 1, 5 and 10% *C. citratus* were 38.4, 75.2 and 100%, respectively. Of the seven essential oils tested, *Cx. quinquefasciatus* was most susceptible to *C. citratus*; the LC_{50} ranged from 2.2 to 20.8% 24 hours after exposure. *C. citratus*, *C. nardus*, *S. aromaticum*, *Ca. odorata*, *O. basilicum*, *E. citriodora* and *Ci. sinensis* gave LC_{50} values of 2.2, 4.1, 5.0, 8.8, 11, 12.2 and 20.8%, respectively.

The KT_{50} values, mortality rates and susceptibilities of *An. dirus* adult mosquitoes are shown in Table 3. *An. dirus* adult mosquitoes were resistant to all seven essential oils at 1% and 5% concentration; the mortality rates ranged from 23.2% to 92.8% with the 1% essential oils and from 40.0% to 97.6% with the 5% essential oils. The mortality rates with the 10% essential oils ranged from 76.8% to 100%. *An. dirus* mosquitoes were resistant to 10% *E. citriodora*, *Ci. sinensis*, *O. basilicum* and *Ca. odorata* and sensitive to 10% *C. nardus*, *S. aromaticum* and *C. citratus*. There were significant differences in mean mortality rates by treatment using ANOVA ($p<0.05$). *C. citratus* gave KT_{50} and mortality rates of 2.39 minutes and 92.8% (at 1%), <1 minute and 97.6% (at 5%) and < 1 minute and 100% (at 10%), respectively, against *An. dirus*. The LC_{50} for the 10% essential oils ranged from <0.1% to 5.0%. Fig 3 shows the knockdown rates for the essential oils at concentrations of 1, 5 and 10%. The seven essential oils gave knock down rates from 0 to 100% (at 1%), 20.8 to 100% (at 5%) and 70 to 100% (at 10%) against *An. dirus* female mosquitoes.

DISCUSSION

Our study found *C. citratus* oil has insecticidal activities against various arthropods. Previous research by Hanifah *et al* (2011) demonstrated *C. citratus* extract has more acaricidal activity against *Dermatophagoides farina* and *D. pteronyssinus* than *Azadirachta indica* at 50% concentration. Samarasekera *et al* (2006) found *C. citratus* oil had good knockdown and mortality activity against adult *M. domestica* at LD_{50} of 1.71 g in Sri Lanka. Senthilkumar *et al* (2009) reported lethality varied by type of mosquito and extract: *E. globulus*, *C. citratus*, *Artemisia annua*, *Justicia gendarussa*, *Myristica fragrans*, *Annona squamosa*, and *Centella asiatica* were found to be most effective against *An. stephensi.*

Several studies have also focused on lemongrass oil for controlling mosquitoes as a larvicide and a repellent with varied results. *C. citratus* essential oil has been studied, showing toxicity against *Cx. quinquefasciatus* larvae with a LC_{50} value of 24 mg/l (Nazar *et al*, 2009) giving 100% protection for up to 5 hours at a concentration of 5.0 mg/cm^2 (Pushpanathan *et al*, 2006). *C. citratus* and *Lippia sidoides* had larvicidal activity against *Ae. aegypti* causing 100% mortality at a concentration of 100 ppm similar to *O. gratissimum* (Cavalcanti *et al*, 2004). Mgbemena (2010) found the essential oil of *O. gratissimium* had greater larvicidal activity than *C. citratus.* Purwal *et al* (2010) evaluated the activity of *C. citratus* and *Mentha piperita* essential oils in combination against *Pediculus humanus* Capitis and found a mean time to death of 60 minutes. Sukumar *et al* (1991) found *C. citratus* caused significant growth inhibition and mortality during later developmental stages of *Ae. aegypti* mosquitoes. *C. citratus* may be included in disease vector control programs, it may be obtained easily and at low cost. *C. citratus* oil may be considered an effective insecticide against mosquitoes in Thailand, and could be used instead of chemical insecticides.

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