POTENTIAL LARVICIDAL AND PUPACIDAL ACTIVITIES OF HERBAL ESSENTIAL OILS AGAINST *CULEX QUINQUEFASCIATUS* SAY AND *ANOPHELES MINIMUS* (THEOBALD)

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Abstract. The larvicidal and pupacidal effects of eight herbal essential oils were tested against third instar (L_3), fourth instar (L_4), and pupal stages of *Culex quinquefasciatus* and *Anopheles minimus*. Probit analysis was used to analyze the data. The larval mortality was recorded at 1, 5, 10, 30, and 60 minutes, and 24 hours. Pupal mortality was also recorded at 24 hours intervals for 96 hours when completed mortality was achieved. Citronella grass oil #2 (*Cymbopogon nardus*) proved to have the greatest toxicity against 3rd instar *Cx. quinquefasciatus* and *An. minimus* with LT_{50} at 1.2 and <0.2 minute, respectively. It exhibited a high level of effectiveness against 4th instar *Cx. quinquefasciatus* (LT_{50} at 5.1 minutes) and *An. minimus* (LT_{50} at 0.9 minute). Regarding pupacidal activity, *Cx. quinquefasciatus* and *An. minimus* pupae were susceptible to Lemon grass oil (LT_{50} at 1.2 hours) and Citronella grass oil #1 (LT_{50} at 0.6 hour), respectively. These oils had larvicidal and pupacidal activities with 100% mortality against L_3, L_4 and pupal stage *Cx. quinquefasciatus* at 10 minutes, 30 minutes and 24 hours. They also caused 100% mortality of *An. minimus* at 5 minutes (L_3, L_4), and 24 hours (pupal stage).

Key words: essential oil, larvicide, pupacide, *Anopheles minimus*, *Culex quinquefasciatus*

INTRODUCTION

Larviciding is one approach to vector control carried out at breeding centers of the vectors (Mohan and Ramaswamy, 2007). This is a successful way of reducing mosquito densities before they emerge as adults. Larval stages breed in water and are more easily dealt with in this habitat; therefore, they are attractive targets for pesticides (Chowdhury *et al*, 2008). They are killed before they disperse to human habitations. Larvae, unlike adult mosquitoes, cannot change their behavior to avoid mosquito control measures (Killeen *et al*, 2002). Larviciding depends largely on the use of organophosphate synthetic chemical insecticides (*eg*, temephos, fenthion) and insect growth regulators (*eg*, diflubenzuron, methoprene) (Ali *et al*,

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1995). In the past, vectors formed resistance to synthetic insecticides, which were expensive and not well accepted by the community (Brown, 1986).

During the last decade, there have been attempts at development of alternative, environmentally friendly and sustainable approaches for mosquito control using natural products with greater target specificity, lower bioaccumulation properties and reduction of malignancy in non-target animals (Benner, 1993). These natural products utilized as mosquito insecticides limit the environmental impact of pesticides due to shorter latency, which may be beneficial for preventing the evolution of resistance (Hardin and Jackson, 2009). There have been no reports of resistance by pests and vectors against botanicals (Madhumathy *et al*, 2007). Active ingredients from many plants have been recognized, isolated, purified and formulated as insecticides. The control of mosquito larvae using indigenous medicinal plants is beneficial in developing countries, such as Thailand and its Southeast Asian neighbors. Many studies have shown chemicals from indigenous plants in Thailand have insecticidal properties against mosquitoes (Komalamisra *et al*, 2005; Promsiri *et al*, 2006; Tawatsin *et al*, 2006).

The purpose of this study was to investigate the potential for eight herbal essential oils, prepared as recommended by KMITL's laboratory, of being used against the immature stages of *Culex quinquefasciatus* and *Anopheles minimus*.

MATERIALS AND METHODS

Mosquitoes

In this study, *Cx. quinquefasciatus* and *An. minimus,* were used. Both species of mosquito were raised in the laboratory of the Entomology and Environment Program, Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Lad Krabang (KMITL), Bangkok. Larvae were reared in plastic trays (size 30x35x5 cm) containing 2 liters tap water and fed on fine fish food. The mosquitoes were maintained and all experiments carried out at 26-28ºC with 70-80% relative humidity. Adult mosquitoes were maintained in cages (size 30x30x30 cm) and continuously provided with 10% sucrose. On Days 5-7, the females were given a blood meal via artificial membrane method. A few days after the blood meal, the gravid mosquitoes laid their eggs. Third and fourth instar larvae and pupal stages, were used for the experiments.

Plant materials

Eight herbal essential oils (10%) in ethyl alcohol were used in this study (Table 1). These oils were provided by the medicinal plant laboratory of KMITL. All formulations were kept at room temperature before being testing.

Bioassay procedures

The test procedures were done according to World Health Organization (1981). One milliliter of tested oil was added to 99 ml distilled water in a 250 ml plastic cup, which was shaken lightly to ensure a homogeneous test solution. Ten specimens each of immature mosquitoes, L_3, L_4, and pupal stages of both *An. minimus* and *Cx. quinquefasciatus* were divided into respective groups and placed in cups. No food was provided during the treatment. Larval mortality rates were recorded at 1 minute intervals for 5 minutes, then 10 minutes intervals for 60 minutes, and then at 24 hours. The pupae were observed for 96 hours for mortality. Larvae were considered dead if they were incapable of rising to the surface or did not show the char-

Table 1
List of herbal essential oils tested in this study.

No.	Common name	Botanical name	Family	Therapeutic property
28 :	Citronella grass#1 oil	*Cymbopogon nardus* L.	Poaceae	Antiseptic, bactericidal, deodorant, diaphoretic, parasitic, tonic, stimulant, insecticide.
37 :	Clove#1 oil	*Syzygium aromaticum* L.	Myrtaceae	Analgesic, antiseptic, antispasmodic, anti-neuralgic, carminative, anti-infectious, disinfectant, stimulant, stomachic, uterine, tonic, insecticide.
38 :	Clove#2 oil	*Syzygium aromaticum* L.	Myrtaceae	Analgesic, antiseptic, antispasmodic, anti-neuralgic, carminative, anti-infectious, disinfectant, stimulant, stomachic, uterine, tonic, insecticide.
39 :	Citronella grass#2 oil	*Cymbopogon nardus* L.	Poaceae	Antiseptic, bactericidal, deodorant, diaphoretic, parasitic, tonic, stimulant, insecticide.
41 :	Eucalyptus oil	*Eucalyptus citriodora* Hook	Myrtaceae	Antifungal, antimicrobial, antiseptic, anti-inflammatory, bactericidal
42 :	Lemon grass oil	*Cymbopogon citratus* DC. Stapf	Poaceae	Analgesic, antifungal, anti-inflammatory, antiseptic, antiviral, bactericidal, digestive, febrifuge, tonic, insecticidal
44 :	Sweet basil oil	*Ocimum basilicum* L.	Lamiaceae	Antiemetic, antiseptic, antispasmodic, carminative, cephalic, expectorant, immune support, insecticide
45 :	Ylang-ylang oil	*Cananga odorata* Lamk.	Annonaceae	Antidepressant, antiseborrheic, antiseptic, aphrodisiac, hypotensive, nervine and sedative.

acteristic dicing reaction when the water was disturbed (Tiwary *et al*, 2007). The mean mortality number were recorded. Each experiment was performed in five replicates with a simultaneous control (1 ml 70% ethanol in 99 ml water).

LT_{50} values (lethal time for 50% mortality) were calculated using probit analysis. The mortality data was analyzed by Duncan's multiple range test using SPSS for Windows (version 16.0).

RESULTS

The LT_{50} values for the eight herbal essential oils against third and fourth instar larvae and pupal stage of *Cx. quinquefasciatus* and *An. minimus* are shown in Tables 2 and 3. These clearly indicate Citronella grass *#2* oil (no. 39) exhibited the highest toxicity against L_3 and L_4 *Cx. quinquefasciatus* and *An. minimus*. The LT_{50} values for 3rd instar *Cx.*

Table 2
LT_{50} values of eight herbal essential oils against third and fourth instars of *Cx. quinquefasciatus* and *An. minimus*.

No. of herbal essential oils	LT_{50} (min)			
	3rd instar		4th instar	
	Cx. quinquefasciatus	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*
28: Citronella grass #1 oil	33.1 (31.0-35.4)	9.5 (8.9-10.3)	44.0 (41.5-46.7)	19.2 (17.3-21.3)
37: Clove #1 oil	1.8 -	5.8 (5.3-7.5)	22.6 -	5.8 -
38: Clove #2 oil	21.8 (17.6-26.6)	20.6 (16.8-25.3)	281.4 -	236.6 (216.1-263.8)
39: Citronella grass #2 oil	1.2 (0.8-1.6)	<0.2 (0.0-0.2)	5.1 -	0.9 (0.6-1.1)
41: Eucalyptus oil	11.7 (5.0-19.6)	6.5 (6.0-7.2)	28.3 (26.6-29.9)	12.4 (5.8-26.2)
42: Lemon grass oil	281.4 -	4.7 (4.4-5.0)	14.8 (12.1-18.1)	14.3 (12.8-16.0)
44: Sweet basil oil	964.8 (<47.1-1,192.6)	51.7 (45.8-59.8)	1,066.6 (381.7-1,239.5)	51.6 (47.0-57.9)
45: Ylang-ylang oil	1,032.3 -	92.9 (74.6-139.2)	1,033.7 (475.7-1,212.0)	137.1 -

LT_{50}, Lethal time for 50% mortality at 95% confidence limit

quinquefasciatus and *An. minimus* were 1.2 and <0.2 minute, respectively, and the LT_{50} values for L_4 instars were 5.1 and 0.9 minutes, respectively. Ylang-ylang oil (no. 45) exhibited the lowest activity against L_3 *Cx. quinquefasciatus* and *An. minimus*, of 1,032.3 and 92.9 minutes, respectively. The essential oils which exhibited the least toxicity against L_4 *Cx. quinquefasciatus* and *An. minimus* were Sweet basil oil (no. 44) with an LT_{50} of 1,066.6 minutes, and Clove #2 oil (no. 38) with an LT_{50} of 236.6 minutes. The essential oil most active against *Cx. quinquefasciatus* pupae was Lemon grass oil (no. 42) with an LT_{50} of 1.2 hour. Citronella grass #1 oil (no. 28) had the greatest LT_{50} level against *An. minimus* pupae of 0.6 hour.

Larvicidal activity of the eight herbal essential oils against 3rd instars 24 hours after exposure, was 98-100% for *Cx. quinquefasciatus* and 100% for *An. minimus* (Table 4). Citronella grass #2 oil (no. 39) gave 100% mortality against 3rd instar *An. minimus* and *Cx. quinquefasciatus* at 5 and 10 minutes, respectively. The mortality rates of the eight herbal essential oils against 4th instar of *Cx. quinquefasciatus* and *An. minimus* were 94-100% and 100%, respectively (Table 5). Citronella grass #2 oil (no. 39) exhibited high efficacy against mosquito species, with 100% mortality at 5 minutes against *An. minimus* and at 30 minutes against *Cx. quinquefasciatus*.

Data regarding the pupacidal activity of the essential oils against the two spe-

Table 3
LT_{50} values for eight herbal essential oils against the pupal stage of *Cx. quinquefasciatus* and *An. minimus*

No. of herbal essential oils	LT50 (hr)	
	Cx. quinquefasciatus	*An. minimus*
28: Citronella grass #1 oil	1.5	0.6
	(1.3-2.0)	(0.5-0.7)
37: Clove #1 oil	25.1	22.9
	-	-
38: Clove #2 oil	28.3	24.5
	(25.5-31.6)	-
39: Citronella grass #2 oil	21.4	0.9
	-	(0.9-1.0)
41: Eucalyptus oil	11.4	0.8
	(7.0-29.2)	-
42: Lemon grass oil	1.2	1
	(1.0-1.4)	(0.9-1.1)
44: Sweet basil oil	28.7	1
	-	(0.9-1.2)
45: Ylang-ylang oil	23.2	1.5
	-	(1.3-1.7)

LT50, Lethal time for 50% mortality at 95% confidence limit

cies of mosquitoes is presented in Table 6. Six of eight essential oils gave 100% mortality against *An. minimus* pupae at 24 hours, but Clove #1 oil (no. 37) and Clove #2 oil (no. 38) did not give 100% mortality by 24 hours, but all herbal essential oils induced 100% mortality 48 hours after exposure. For *Cx. quinquefasciatus* pupae six herbal essential oils induced 100% mortality, by 72 hours, but Sweet basil oil (no. 44) and Ylang-ylang oil (no. 45) did not give 100% mortality.

DISCUSSION

Larvicide application is a successful way of reducing mosquito densities in their habitats before they emerge as adults. In this study, eight herbal essential oils had a highly toxic effect against L_3, L_4 instars and pupal stages of both *Cx. quinquefasciatus* and *An. minimus.*

Cymbopogon sp essential oils have larvicidal and pupacidal activity against immature *Cx. quinquefasciatus* and *An. minimus* mosquitoes. Citronella grass #2 oil (*C. nardus*) proved to have insecticidal efficacy against both L_3 and L_4 instars and Lemon grass oil (*C. citratus)* and Citronella grass oil #1 (*C. nardus*) exhibited toxicity against pupae. Citronella grass #2 oil had greater larvicidal activity against third instars at 1 minute ($p < 0.05$) and fourth instars at 5 minutes ($p < 0.05$). It also gave 100% mortality against *Cx. quinquefasciatus* and *An. minimus*, at 10 minutes and 5 minutes (L_3) and at 30 minutes and 5 minutes (L_4), respectively. *C. nardus* was found to have larvicidal action against late 3rd instar *Cx. quinquefasciatus* larvae (Ranaweera

Table 4
Larvicidal activity of herbal essential oils against the 3rd instar larvae of *Cx. quinquefasciatus* and *An. minimus*.

No. of herbal essential oils	% Mortality±SD / Time (min, hr)											
	1 min		5 min		10 min		30 min		60 min		24 hr	
	Cx. quinquefasciatus	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*
28: Citronella grass#1 oil	0.0$^{c\,1}$	0.0^{b}	0.0^{c}	0.0^{d}	12.0±13.0^{d}	56.0±33.6^{b}	18.0±13.0^{c}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{ns}
37: Clove#1 oil	24.0±32.9^{b}	0.0^{b}	98.0±4.5^{a}	26.0±23.0^{c}	98.0±4.5^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
38: Clove#2 oil	0.0^{c}	0.0^{b}	0.0^{c}	22.0±31.9^{cd}	76.0±11.4^{c}	38.0±41.5^{bc}	86.0±8.9^{b}	84.0±11.4^{b}	88.0±11.0^{b}	98.0±4.5^{a}	100.0±0.0^{a}	100.0±0.0
39: Citronella grass#2 oil	74.0±5.5^{a}	92.0±13.0^{a}	90.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
41: Eucalyptus oil	0.0^{c}	0.0^{b}	60.0±25.5^{b}	18.0±21.7^{cd}	88.0±8.4^{b}	98.0±4.5^{a}	92.0±8.4^{b}	100.0±0.0^{a}	96.0±8.9^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
42: Lemon grass oil	0.0^{c}	0.0^{b}	0.0^{c}	76.0±32.9^{b}	0.0^{e}	100.0±0.0^{a}	0.0^{d}	100.0±0.0^{a}	2.0±4.5^{c}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
44: Sweet basil oil	0.0^{c}	0.0^{b}	0.0^{c}	0.0^{d}	0.0^{e}	24.0±16.7^{c}	0.0^{d}	24.0±16.7^{c}	0.0^{c}	78.0±17.9^{b}	98.0±4.5^{a}	100.0±0.0
45: Ylang-ylang oil	0.0^{c}	0.0^{b}	0.0^{c}	0.0^{d}	0.0^{e}	0.0^{d}	0.0^{d}	6.0±13.4^{d}	0.0^{c}	12.0±13.0^{c}	98.0±4.5^{a}	100.0±0.0
Control (70% Ethyl alcohol)	0.0^{c}	0.0^{b}	0.0^{c}	0.0^{d}	0.0^{e}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{c}	0.0^{d}	0.0^{b}	0.0
CV%	107.5%	44.8%	33.0%	72.8%	16.7%	34.4%	14.3%	12.5%	9.7%	10.4%	2.5%	NA

[1]Means in each column followed by the same letter are not significantly different (p>0.05, by one-way ANOVA and Duncan's multiple range test).
ns , not significant

Table 5
Larvicidal activity of herbal essential oils against the 4th instar larvae of *Cx. quinquefasciatus* and *An. minimus*.

Herbal essential oils	% Mortality±SD / Time (min, hr)											
	1 min		5 min		10 min		30 min		60 min		24 hr	
	Cx. quinquefasciatus	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*
28: Citronella grass#1 oil	0.0^{ns}	0.0$^{b\ 1}$	0.0^{b}	0.0^{d}	0.0^{c}	40.0±18.7^{c}	20.0±20.0^{c}	82.0±17.9^{b}	80.0±15.8^{b}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0 ^{ns}
37: Clove#1 oil	0.0	0.0^{b}	0.0^{b}	86.0±11.4^{b}	12.0±17.9^{bc}	98.0±4.5^{a}	96.0±5.5^{a}	100.0±0.0^{a}	98.0±4.5^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
38: Clove#2 oil	0.0	0.0^{b}	0.0^{b}	0.0^{d}	0.0^{c}	0.0^{d}	0.0^{d}	0.0^{c}	2.0±4.5^{c}	0.0^{c}	100.0±0.0^{a}	100.0±0.0
39: Citronella grass#2 oil	0.0	22.0±19.2^{a}	74.0±11.4^{a}	100.0±0.0^{a}	86.0±11.4^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
41: Eucalyptus oil	0.0	0.0^{b}	0.0^{b}	52.0±4.5^{c}	0.0^{c}	80.0±14.1^{ab}	76.0±25.1^{b}	92.0±8.4^{ab}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
42: Lemon grass oil	0.0	0.0^{b}	0.0^{b}	0.0^{d}	18.0±30.3^{b}	68.0±8.4^{b}	98.0±4.5^{a}	94.0±5.5^{ab}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
44: Sweet basil oil	0.0	0.0^{b}	0.0^{b}	0.0^{d}	0.0^{c}	40.0±54.8^{c}	0.0^{d}	92.0±17.9^{ab}	2.0±4.5^{c}	90.0±22.4^{a}	100.0±0.0^{a}	100.0±0.0
45: Ylang-ylang oil	0.0	0.0^{b}	0.0^{b}	0.0^{d}	0.0^{c}	0.0^{d}	0.0^{d}	6.0±13.4^{c}	0.0^{c}	12.0±13.0^{b}	94.0±8.9^{b}	100.0±0.0
Control (70% Ethyl alcohol)	0.0	0.0^{b}	0.0^{b}	0.0^{d}	0.0^{c}	0.0^{d}	0.0^{d}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0
CV%	NA	276.5%	48.7%	16.3%	100.9%	44.8%	26.6%	16.9%	12.0%	13.6%	3.6%	NA

[1]Means in each column against each mosquito species followed by the difference letter are significantly different ($p<0.05$, by one-way ANOVA and Duncan's multiple range test).
ns, not significant

Table 6
Larvicidal activity of herbal essential oils against the pupal stages of *Cx. quinquefasciatus* and *An. minimus*.

Herbal essential oils	% Mortality±SD / Time (hr)									
	1 hr		24 hr		48 hr		72 hr		96 hr	
	Cx. quinquefasciatus	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*	*Cx. quinquefasciatus*	*An. minimus*
28: Citronella grass#1 oil	36.0+18.2$^{b\ 1}$	98.0+4.5^{a}	100.0+0.0^{a}	100.0+0.0^{a}	100.0+0.0^{a}	100.0+0.0^{ns}	100.0+0.0^{a}	100.0+0.0^{ns}	100.0+0.0^{a}	100.0+0.0^{ns}
37: Clove#1 oil	0.0^{c}	0.0^{c}	42.0+8.4^{c}	58.0+28.6^{b}	100.0+0.0^{a}	100.0+0.0	100.0+0.0^{a}	100.0+0.0	100.0+0.0^{a}	100.0+0.0
38: Clove#2 oil	0.0^{c}	0.0^{c}	34.0+5.5^{c}	46.0+41.6^{b}	98.0+4.5^{a}	100.0+0.0	100.0+0.0^{a}	100.0+0.0	100.0+0.0^{a}	100.0+0.0
39: Citronella grass#2 oil	0.0^{c}	76.0+23.0^{b}	70.0+18.7^{b}	100.0+0.0^{a}	100.0+0.0^{a}	100.0+0.0	100.0+0.0^{a}	100.0+0.0	100.0+0.0^{a}	100.0+0.0
41: Eucalyptus oil	2.0+4.5^{c}	90.0+10.0^{a}	100.0+0.0^{a}	100.0+0.0^{a}	100.0+0.0^{a}	100.0+0.0	100.0+0.0^{a}	100.0+0.0	100.0+0.0^{a}	100.0+0.0
42: Lemon grass oil	58.0+16.4^{a}	98.0+4.5^{a}	100.0+0.0^{a}	100.0+0.0^{a}	100.0+0.0^{a}	100.0+0.0	100.0+0.0^{a}	100.0+0.0	100.0+0.0^{a}	100.0+0.0
44: Sweet basil oil	6.0+5.5^{c}	96.0+5.5^{a}	92.0+11.0^{a}	100.0+0.0^{a}	96.0+8.9^{a}	100.0+0.0	96.0+8.9^{a}	100.0+0.0	96.0+8.9^{a}	100.0+0.0
45: Ylang-ylang oil	34.0+11.4^{b}	64.0+18.2^{b}	90.0+12.2^{a}	100.0+0.0^{a}	98.0+4.5^{a}	100.0+0.0	98.0+4.5^{a}	100.0+0.0	98.0+4.5^{a}	100.0+0.0
Control (70% Ethyl alcohol)	0.0^{c}	0.0^{c}	0.0^{d}	0.0^{c}	0.0^{b}	0.0	0.0^{b}	0.0	0.0^{b}	0.0
CV%	64.9%	19.4%	13.5%	22.7%	4.4%	NA	4.0%	NA	4.0%	NA

[1]Means in each column against each mosquito species followed by the difference letter are significantly different ($p<0.05$, by one-way ANOVA and Duncan's multiple range test).
ns , not significant

and Dayananda, 1996) and adulticidal activity or repellant activity against *Ae. aegypti* adults (Zaridah *et al,* 2006). Citronella grass #2 oil (no. 39) exhibited less pupacidal activity against pupal stages than Lemon grass oil (no. 42) and Citronella grass #1 oil (no. 28). These oils were found to have a higher rate of pupacidal activity against *Cx. quinquefasciatus* (LT_{50} at 1.2 hour) and *An. minimus* (LT_{50} at 0.6 hour), respectively. Sukumar *et al* (1991) reported *C. citratus* caused significant growth inhibition and mortality in later developmental stages of *Ae. aegypti*. However, four of the eight essential oils tested exhibited pupacidal activity against *An. minimus* pupae (90-98%) at 1 hour ($p > 0.05$). Five of the eight essential oils tested exhibited toxicity against *Cx. quinquefasciatus* pupae (90-100%) at 24 hours ($p > 0.05$) (Table 6). Although Citronella grass #1 oil (no. 28) and Citronella grass #2 oil (no.39) were extracted from the same plant material (*Cymbopogon nardus*) they had different inert ingredients in each formulations. Thus, they had the different results for LT_{50} and % mortality.

Our results agree with those of Amer and Mehlhorn (2006) where herbal essential oils were tested against the larvae and pupae of *Cx. quinquefasciatus* and *An. minimus*, showing anophelines were more sensitive than culicines. Herbal oils toxicity gave an LTs_{50} against *An. minimus* of <0.2 minute (L_3), 0.9 minute (L_4) and < 1 hour (pupal stage).

Cymbopogon nardus and *C. citratus* showed larvicidal and pupacidal activity against both *Cx. quinquefasciatus* and *An. minimus* in the laboratory. These essential oils might be used to control vectors in the endemic areas without damaging the environment. These larvicides may best be used in small breeding places, such as containers and coolers, where the water is stagnant (Ansari *et al,* 2005) because it is easier to kill larvae in stagnant water than to kill adult mosquitoes. Field trials are needed to assess the efficacy, cost-effectiveness and effects against non-target organisms of these essential oils.

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