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Ovicidal and adulticidal activities of *Cymbopogon citratus* (DC.) Stapf and *Illicium verum* Hook. f. against *Aedes aegypti* (Linn.)

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Abstract The hatching inhibition and knockdown rates against *Aedes aegypti* of two essential oils (EOs): *Cymbopogon citratus* DC. Stapf and *Illicium verum* Hook F was evaluated. The efficacy of each of these EOs, at 1, 5, 10% emulsion in water, stabilized by tween 60®, was compared to that of 1% *w*/*v* temephos and 1% *w*/*w* cypermethrin (common, harmful synthetic insecticides). Topical and contact assays showed that 10% *C. citratus* and 10% *I. verum* emulsions were the most effective in inhibiting the hatching of mosquito eggs (100%) after 48 hours of exposure. Moreover, they were also the most toxic against mosquito adults (100% mortality) after 24 hours of exposure. This study also established that tween 60® had no effect on hatching inhibition or mortality rate of treated *Aedes aegypti* mosquitoes. All EO emulsions were more potent than temephos and cypermethrin against these mosquito species. Coupling this higher efficacy with no or benign known side effects of natural EOs, it can be concluded that 10% *C. citratus* and 10% *I. verum* emulsions are better alternatives than temephos and cypermethrin for a mosquito control program at the present time.

Keywords: *Aedes aegypti*, *Cymbopogon citratus* (Stapf.), *Illicium verum* Hook. F.

Introduction

Aedes aegypti (Linn.) is considered a destructive insect in medicine and public health. Even though it is found mostly in tropical regions, it can live anywhere in the world. Their habitats are such as waterlogging area, hollow, and water tank. Mosquito eggs are hardy, i.e., they can stay viable through a drought (Silvério *et al*., 2020; Reinhold *et al*., 2018). Most importantly, it is a vector of many serious human diseases, such as dengue, yellow fever, chikungunya, and Zika virus. Dengue infects humans easily, inflicting 50-100 million people annually (Tantawichien and Thisyakorn, 2017; WHO, 2020a; Wilder-Smith *et al*., 2019). An estimate of 2.5 billion people being at risk around the world annually, and the infection rate has been increasing rapidly.

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Dengue is believed to penetrate into capillaries, causing thrombocytopenia and possibly eventual death (WHO, 2020a). Chikungunya is another destructive virus that causes pain in the joints and muscles in the early stage of infection, whereas Zika virus affects the nervous system, causing Guillain-Barré Syndrome and meningoencephalitis. Pregnant women infected with Zika may give birth to microcephalic child, baby having an abnormally small head (Chansang *et al*., 2018; WHO, 2020a; WHO, 2020b).

The most popular prevention method against these diseases has been to destroy the vector population with effective synthetic chemicals. Widely used ones are organophosphates, carbamates, and pyrethroids (Nicolopoulo *et al*., 2016; Kandel *et al*., 2019; WHO, 2020c). However, after these chemicals have been used extensively and effectively to control insect vectors in a region, the insects developed resistance to them (Demok *et al*., 2019; Collins *et al*., 2019; Hamid *et al*., 2018). Nowadays, insect vectors all around the world are resistant to these insecticides. Not only being ineffective at the present days, these previously effective synthetic chemicals also cause serious damages to human's nervous and respiratory systems (Demok *et al*., 2019; Nicolopoulo *et al*., 2016). Moreover, they persisted in the environment for a long time, not biologically degraded quickly like natural compounds (Aungtikun and Soonwera, 2021; Isman, 2017).

Therefore, safe and sustainable alternatives have been explored. Good candidates have been essential oils (EOs) extracted from medicinal herbs because they are not toxic to humans and animals and do not leave persistent residue in the environment. In addition, the following EOs have already been reported to repel or destroy some mosquito vectors (Chansang *et al*., 2018; Nicolopoulo *et al*., 2016; Sarma *et al*., 2020; Pavela and Benelli, 2016): *Murraya koenigii*, *Ficus benghalenssis*, *Hottuynia cordata*, *Callistemon linearis*, *Psidium guajava*, *Eupatorium odoratum*, *Ageratum conyzoids*, *Zingeber offcinale*, *Polyalthia longifolia*, *Spondias pinata*, *Lantana camara*, *Hamalomena aromatica*, *Ocimum sanctum*, *Eucalyptus maculatus*, *Lippia alba*, *Mentha piperita*, *Azadirachta indica*, *Allium sativum*, *Plumeria rubra*, *Cyperus rotundus*, *Alpinia galanga*, and *Cinnamomum alangum*. However, the ovicidal and adulticidal activities of *Cymbopogon citratus* and *Illicium verum* against *Aedes aegypti* have not been reported in the literature yet. Therefore, this study attempted to evaluate the efficacy of these EOs against all life stages of *Ae. aegypti*.

Materials and methods

Mosquito rearing

Colonies of a mosquito species, *Ae. aegypti* were provided by the Entomological Laboratory, Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. The colonies were kept under the following conditions: 27.0±3.3 ℃, 72.5±1.5%RH, and 12-h light and 12-h dark lighting period. The eggs hatched out into around 200 larvae in a plastic box (28×35×4 cm^3) filled with 2000 ml of drinking water. They fed on fish food (OPTIMUM®, 32% protein). Fourteen-day-old larvae developed into pupae, then 150 pupae were collected and put in a 250 ml plastic cup containing 200 ml of drinking water. The cup was transferred into a mosquito cage (30×30×30 cm^3). No food was fed to the pupae in the cage. Two-day-old pupae developed into adult mosquitoes.The adults fed on 5% glucose solution mix 1% multivitamin syrup solution in drinking water in soaked cotton pads. When they were 5 days old, female mosquitoes were given blood as food for 60 min by an artificial membrane method (Aungtikun and Soonwera, 2021; Cotchakaew and Soonwera, 2018). Three days afterwards, the gravid mosquitoes laid eggs. An ovicidal bioassay was performed on most of the eggs, but some of the eggs were hatched into adults, and two-day-old female adults were subjected to an adulticidal activity assay.

Essential oils

Plant materials used in this study were fresh stems of *Cymbopogon citratus* DC. Stapf gathered from local plants in Samutphakan province, Thailand. Dry fruits of *Illicium verum* Hook. F. were purchased from Vejpong Pharmacy (hock ann tung) Co. Ltd, Thailand. Specimens of the two plants were positively identified by a plant taxonomist from the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. The fresh stems and dry fruits were cleaned, cut into small pieces, and extracted for 5 h for their EOs by a water distillation method. After the distillation was completed, EOs were collected from the separating funnel, stored in airtight bottles, and kept at 4 ℃ for later experiments. All EO formulations (solutions with different concentration of an EO) were prepared by diluting the stock extracted EO solution with drinking water containing 3.5% tween 60® to the desired concentration.

Positive and negative controls

- A positive control was cypermethrin (Cyperguard 10 EC®, 10% *w/v* cypermethrin), manufactured by Expert pest system Co. Ltd, Bangkok, Thailand.

- Another positive control was temephos (SaiGPO-1®, 1.0% *w/w* temephos), manufactured by Thailand's Government Pharmaceutical Organization, Thailand. Of note is that temephos was a solid product, hence to use it as a control, 0.01g of it was dissolved in 100 ml of drinking water in a 250 ml plastic cup.

- Tween 60® was the negative control, manufactured by Kao corporation, Japan.

Ovicidal bioassay

The ovicidal bioassay was the dipping method used by Cotchakaew and Soonwera (2018). This experiment was of a completely randomized design. Five replicates of each treatment were run, and the average results were compared to that produced by 1% *w*/*w* temephos. LC_{50} (Lethal Concentration for 50%mortality) were calculated by probit analysis. A Duncan's Multiple Range Test was conducted on the mortality data, with SPSS statistical software for Windows (version 16.0).

Twenty five eggs of each species of mosquitoes were placed in a 250-ml plastic cup containing 99 ml of drinking water. One milliliter of each formulation of *C. citratus* and *I. verum* EOs was added to the cup. After 48 hours, the hatched larvae were counted. Five replicates of the treatment were run, and the results were compared to those produced by temephos and drinking water. The percentage egg inhibition rate was calculated by the following formula:

$$\text{Inhibition rate (\%)} = [NT/NC] \times 100,$$

where NT is the total number of dead eggs (not hatched within 48 hours) and NC is the total number of treated eggs.

World Health Organization (WHO) susceptibility test

Knockdown rate, mortality rate, and susceptibility classification of adulticidal agents were determined by the standard World Health Organization (WHO, 2018) contact method, with a completely randomized design. Five replicates of each treatment were run, and the average results were compared to that produced by 10% *w*/*w* cypermethrin, the positive control. Twenty-five-day-old female mosquitoes (2 days after emergence) unfed with any blood meal were exposed to *C. citratus* and *I. verum* EOs. Two milliliters of either 1 or 5 or 10% concentration of the EO were dropped onto a filter paper (the size of

12×15 cm, Whatman No.1®) which was then placed in the treatment tube (44 mm in diameter and 125 mm in length). After 1 h of exposure, the mosquitoes were transferred to the non-treatment tube containing a plain filter paper to ensure that the filter papers, by themselves, did not affect the assay outcomes. The knockdown rates were recorded at 1, 5, 10, 15, 30, and 60 min, and the mortality rates were recorded 24 h after the start of the exposure. Knockdown and mortality were evident by no movement of any body parts (head, antenna, thorax, wings, legs, abdomen, and other appedages) when the treated mosquito was prodded with a soft brush.
Knockdown rate and mortality rate were calculated by the following formula:

$$\text{Knockdown rate (\%)} = [NT/NC] \times 100,$$

where NT is the total number of knocked-down mosquitoes, and NC is the total number of treated mosquitoes.

KT_{50} (50% knockdown time) and LC_{50} (50% lethal concentration) were calculated by probit analysis. Mortality data were analyzed by Duncan's Multiple Range Test with SPSS for Windows software (version 16.0). Adulticidal agent's susceptibility criteria as classified by WHO (2018) are as follows: 98.0-100% mortality signifies susceptibility (S); 80.0-97.0% mortality signifies possibly resistant that needs confirmation (PR); and less than 80.0% signifies resistant (R).

Results

Ovicidal bioassay

The percentage hatching-inhibition rates against *Ae. aegypti* eggs of *C. citratus* and *I. verum* EOs at 1, 5, and 10% and temephos are listed in Table 1. *C. citratus* and *I. verum* EOs at 10% exhibited a 100 %inhibition rate against *Ae. aegypti* eggs. In addition, at a half of the maximum concentration tested, 5% *C. citratus* and *I. verum* EOs exhibited 80.6 and 91.1% inhibition rates, respectively, and at only a tenth of the maximum concentration, the 1% EOs provided a 47.0 and 63.4% hatching-inhibition rates, respectively. In contrast, temephos showed a much lower toxicity to the eggs of those mosquito species tested, with an inhibition rate of only 34.6%, while tween 60® (the negative control) showed no toxicity at all against mosquito eggs.

Table 1. Ovicidal activities against *Ae. aegypti* eggs of 1%, 5% and 10% *C. citratus* and *I. verum* EOs and 1% *w*/*v* temephos

Treatment	Conc (%)	Inhibition rate ±SD		LC_{50}[1/](%)
		24 h	48 h	
C. citratus	1	64.5 ±1.5[d3/]	47.0 ±4.2[e]	
C. citratus	5	87.5 ±8.0[c]	80.6 ±4.82[c]	1.38
C. citratus	10	100 ±0[a]	100 ±0[a]	
I. verum	1	82.6 ±0.6[c]	63.4 ±3.8[d]	
I. verum	5	93.1 ±5.3[b]	91.1 ±2.5[b]	<1
I. verum	10	100 ±0[a]	100 ±0[a]	
Temephos	1 *w/v*	59.2 ±31.1[e]	34.6 ±19.6[f]	ns[2/]
Tween 60®		0[f]	0[g]	ns
df_{total}, *F*-test		39	39	
C.V. (%)		60.8	58.8	

[1/]LC_{50}, 50% lethal concentration.
[2/]ns: Could not be determined by Probit analysis
[3/]Mean % mortality rates followed by the same letter in the same column are not significantly different at $p < 0.05$ (one-way ANOVA and Duncan's multiple range test).

World Health Organization (WHO) susceptibility test

Knockdown rate and knockdown time, KT_{50}, against females of *Ae. aegypti* of *C. citratus and I. verum* EOs are tabulated in Table 2, while their LC_{50} and WHO susceptility status are listed in Table 3. Both EOs at all 1, 5, and 10% concentrations provided a 100% mortality rate after 1 hr of exposure. However, the more concentrated 5 and 10% *C. citratus* provided a shorter mortality time (after 10 min of exposure) than the less concentrated 1% formulation (after at least 15 min of exposure). Interestingly, *I. verum* provided a 100% mortality rate at all 1, 5, and 10% concentrations after only 15 min of exposure. Based on the KT_{50} of *C. citratus*, 10% concentration provided the shortest mortality time, followed by 5% (2.1 min) and 1% (2.9 min). Regarding LC_{50}, Its LC_{50} was calculated to be 11.92%. Following the same trend, the KT_{50} of 10% *I. verum* was the shortest at 2.1 min, followed by 5% (2.6 min) and 1% (3.1 min), with an LC_{50} of 13.2%. In contrast, the KT_{50} of cypermethrin was 4.3 min, which means that it takes at least two times longer to destroy *Ae. aegypti* females than both EOs at 1%, the lowest concentration tested. As expected, the tween 60® control did not destroy or affect any mosquitoes throughout the experiment.

Table 2. Knockdown rate and KT_{50} against *Ae*. *aegypti* of 1, 5, and 10% *C*. *citratus* and *I*. *verum* EOs and 1% *w*/*w* cypermethrin

Treatment	Conc (%)	Knockdown rate (%) ± SD			KT_{50}[1/]
		5 min	10 min	15 min	
C. citratus	1	60.0 ±4.0[d3/]	99.2 ±1.7[a]	100[a]	2.9 (2.5-3.4)
C. citratus	5	96.8 ±3.3[ab]	100[a]	100[a]	2.1 (1.7-2.6)
C. citratus	10	99.2 ±1.7[a]	100[a]	100[a]	1.9 (1.3-2.4)
I. verum	1	59.2 ±5.2[d]	85.6 ±9.2[c]	100[a]	3.2 (2.8-3.7)
I. verum	5	72.8 ±7.69[c]	95.2 ±4.3[b]	100[a]	2.6 (2.2-3.0)
I. verum	10	85.6 ±2.1[b]	99.2 ±1.7[a]	100[a]	2.2 (1.8-2.6)
Cypermethrin	1*w*/*w*	21.3 ±6.1[e]	50.6 ±6.1[d]	100[a]	4.4 (3.9-5.0)
Tween 60®		0[f]	0[f]	0[b]	ns[2/]
df_{total}, *F*-test		39	39	39	
C.V. (%)		37.06	27.88	27.22	

[1/]KT_{50}, 50% knockdown time
[2/]ns: Could not be determined by Probit analysis
[3/]Mean % mortality rates followed by the same letter in the same column are not significantly different at $p < 0.05$ (one-way ANOVA and Duncan's multiple range test).

Table 3. Mortality rate and LC_{50} against *Ae*. *aegypti* as well as WHO susceptibility status of *C*. *citratus*, *I*. *verum* EOs, and 1% *w*/*w* cypermethrin

Treatment	Conc (%)	Mortality rate (%) ± SD	LC_{50} (%)[1/]	Susceptibility status[2/]
		60 min		
C. citratus	1	100		S
C. citratus	5	100	11.9	S
C. citratus	10	100		S
I. verum	1	100		S
I. verum	5	100	13.2	S
I. verum	10	100		S
Cypermethrin	1 *w*/*w*	100	ns[3/]	S
Tween 60®		0	ns	R

[1/]LC_{50}, 50% lethal concentration.
[2/]S, Susceptibility is defined as 98-100% mortality; RS, Resistance suspected is defined as 80-97% mortality; and R, Resistant, is defined as <80% mortality.
[3/]ns = Could not be determined by Probit analysis.

Discussion

The first point of discussion is that 10% *I*. *verum* EO was more effective than temephos at hatching inhibition of *Ae*. *aegypti* mosquito eggs, most likely because temephos was ingested by the larvae but not the eggs (Ling *et al*., 2013). This conclusion is supported by a finding by Cotchakaew and Soonwera (2018) that 10% *I*. *verum* EO was able to inhibit, at 100% rate, the hatching of

the eggs of *Ae*. *albopictus* (Skuse) and *Anopheles minimus* (Theobald). Moreover, it was also able to inhibit the hatching of housefly eggs at 97.3% rate (Sinthusiri and Soonwera, 2014).

The second point is that our finding that 10% *C*. *citratus* EO was more effective than cypermethrin in knocking down *Ae*. *aegypti* mosquitoes is consistent with a finding by Soonwera and Sittichok, (2020) that *C*. *citratus* EO and geranial provided a high mortality rate against the mosquitoes. Not only providing a higher knockdown rate than cypermetrhin, 10% *C*. *citratus* EO also provided a higher mortality rate.

The final point of discussion is that *C*. *citratus* and *I*. *verum* essential oils are better natural alternatives to cypermethrin because they not only were more effective at the time of this study but also safer to non-targeted organisms since they were natural substances that have been consumed since ancient times as folk medicine (Silvério *et al*., 2020). In modern time, it has been used in perfume industry because it smelled good and was safe for users (Irshad *et al*., 2020). However, a gold standard safety evaluation of these EOs should be conducted before they are released as an alternative commercial product to cypermethrin.

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References

Aungtikun, J. and Soonwera, M. (2021). Impoved adulticidal activity against *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) from synergy. Scientific Reports, 11:4685.

Chansang, A., Champakaew, D., Junkum, A., Jitpakdi, A., Amornlerdpison, D., Aldred, A. K. and Pitasawat, B. (2018). Synergy in the adulticidal efficacy of essential oils for the improvement of permethrin toxicity against *Aedes aegypti* L. (Diptera: Culicidae). Parasites and Vectors, 11:417.

Collins, E., Natasha, M. V., Moussa, S., Abdoul, H. B., James, O., Gena, L., Ryan, E. W., Seth, R. I., Thomas, W. and Louisa, A. M. (2019). The relationship between insecticide resistance, mosquito age and malaria prevalence in *Anopheles gambiae* s.l. from Guinea. Scientific Reports, 9: 8846.

Cotchakaew, N. and Soonwera, M. (2018). "Efficacise of essential oils from Illiciaceae and Zingiberaceae plant as oviposition deterrent, ovicidal, and adulticidal agents agains females *Aedes albopictus* (Skuse) and *Anopheles minimus* (Theobald)". International Journal of Agricultural Technology, 14:631-652.

Demok, S., Endersby-Harshman, N., Vinit, R., Timinao, L., Robinson, L. J., Susapu, M., Makita, L., Laman, M., Hoffmann, A. and Karl, S. (2019). Insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* mosquitoes in Papua New Guinea. Parasites and Vectors, 12:333.

Hamid., P. H., Ninditya, V. I., Prastowo, J., Haryanto, A., Taubert, A. and Hermosilla, C. (2018). Current status of *Aedes aegypti* insecticide resistance development from Banjarmasin, Kalimantan, Indonesia. BioMed Research International. ID 1735358.

Irshad, M., Subhani, M. A., Ali, S. and Hussain, A. (2020). Biological importance of essential oils. Biological Importance of Essential Oils. Retrieved from dio :10.5772/intechopen.87198.

Isman, M. N. (2017). Bridging the gap: Moving botanical insecticides from the laboratory to the farm. Industrial Crops and Products. 110:10-14.

Ling, L. S., Sulaiman, S. and Othman, H. (2013). Laboratory evaluation of temephos, grass infusion, and *Piper aduncum* extracts against the ovipository responses of *Aedes aegypti*. The Journal of Tropical Medicine and Parasitology, 36:15-22.

Kandel, Y., Julia, V., Stacy, D. R., Emily, M., Hae-Na, C., Soumi, M., Joel, J. Cordova., Kalli, J.L. M., Alex, S. M., Aditi, K., Paul, E., Sandra, M., Jiannong, Xu., Michaela, B., Kathryn, A. H. and Immo, A. H. (2019). Widespread insecticide resistance in *Aedes aegypti* L. from New Mexico, U.S.A. PLoS One. 14:e0212693.

Nicolopoulo, S. P., Maipas, S., Kotampasi, C., Stamatis, P. and Hens, L. (2016). Chemical pesticides and human health: the urgent need for a new concept in agriculture. Front. Public Health, 4:1-8.

Pavela, R. and Benelli, G. (2016). Ethnobotanical knowledge on botanical repellents employed in the African region against mosquito vectors - a review. Parasitol. 167:103-108.

Reinhold, J. M., Lazzari, C. R. and Lahondère, C. (2018). Effects of the environmental temperature on *Aedes aegypti* and *Aedes albopictus* mosquitoes: a review. Insects, 9:158.

Sarma, R., Adhikari, K., Mahanta, S. and Khanikor, B. (2020). Twenty essential oils as ovicidal agent against *Aedes aegypti* (Diptera: Culicidae). National Academy Science Letters. Retrieved from doi:10.1007/s40009-020-00923-1.

Silvério, M. R. S., Espindola, L. S., Lopes, N. P. and Vieira, P. C. (2020). Plant natural products for the control of *Aedes aegypti*: the main vector of important arboviruses. Molecules, 25:3484.

Sinthusiri, J. and Soonwera, M. (2014). Oviposition deterrent and ovicidal activities of seven herbal essential oils against female adults of housefly, *Musca domestica* L. Parasitology Research, 113:3015-3022.

Soonwera, M. and Sittichok, S. (2020). Adulticidal activities of *Cymbopogon citratus* (Stapf.) and Eucalyptus globulus (Labill.) essential oils and of their synergistic combinations against *Aedes aegypti* (L.), *Aedes albopictus* (Skuse), and *Musca domestica* (L.). Environmental Science and Pollution Research, 27:20201-20214.

World Health Organization (WHO) (2018). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes – 2nd ed. Geneva: World Health Organization. Retrieved from https://apps.who.int/iris/bitstream/handle/10665/250677/9789241511575eng.pdf.

World Health Organization (WHO) (2020a). Dengue situation update2020. Retrieved from http://iris.wpro.who.int/handle/10665.1/14184.
World Health Organization (WHO) (2020b). What is dengue fever?. Retrieved from http://breakdengue.org/dengue-fever.
World Health Organization (WHO) (2020c). Insecticide resistance. Retrieved from www.who.int/malaria/areas/vectors-control/insectide-resistance/en.

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