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Efficacies of four plant essential oils as larvicide, pupicide and oviposition deterrent agents against dengue fever mosquito, *Aedes aegypti* Linn. (Diptera: Culicidae)

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ABSTRACT

Objective: To evaluate larvicidal, pupicidal and oviposition deterrent activities of four plant essential oils from *Alpinia galanga* (L.) Willd rhizome, *Anethum graveolens* L. (*An. graveolens*) fruit, *Foeniculum vulgare* Mill. fruit, and *Pimpinella anisum* L. fruit against *Aedes aegypti* (*Ae. aegypti*). **Methods:** Four essential oils at 1%, 5% and 10% concentrations were assessed for insecticidal activity against larvae and pupae of *Ae. aegypti*, following the procedure of a dipping method assay. Oviposition deterrent activity of four essential oils was evaluated on gravid female of *Ae. aegypti* by a dual-choice oviposition bioassay. **Results:** The results revealed that *An. graveolens* oil provided the strongest larvicidal activity against *Ae. aegypti* among four tested plant essential oils with the highest mortality rate of 100% and LC_{50} value of -0.3%. From the pupicidal experiment, *An. graveolens* also showed the highest toxicity against *Ae. aegypti* pupae with the highest mortality rate of 100% at 72 h and LC_{50} value of 2.9%. In addition, 10% *An. graveolens* had an oviposition deterrent effect against *Ae. aegypti* with effective repellency of 100% and an oviposition activity index of –1.0. **Conclusions:** *An. graveolens* oil has a good potential as a larvicidal, pupicidal and oviposition deterrent agent for controlling *Ae. aegypti*.

1. Introduction

Mosquitoes (Culicidae: Diptera) are a serious worldwide threat for humans and animals. They are vectors of many serious pathogens and parasites including dengue, Zika virus, malaria and filariasis[1]. *Aedes aegypti* (L.) (*Ae. aegypti*) that inhabits the tropical and subtropical zones carries arbovirus, and is generally known to be a vector of dengue and chikungunya. *Ae. aegypti* females are anthropophilic, humans are their preferred hosts and thus at risk of being attacked by them[2,3]. Studies have suggested that most females of *Ae. aegypti* may spend all of their lives in or around the houses that they have emerged as an adult. *Ae. aegypti* transmits dengue virus to susceptible humans[4]. It has been estimated recently that 3.9 billions of people in 128 countries are at risk of acquiring dengue and 390 million dengue infections occur every year, of which 294 millions clinically manifest the symptoms[5]. Severe dengue is a relatively rare but serious complication of dengue infection is manifested as plasma leaking, fluid accumulation, respiratory distress, severe bleeding or organ impairment[4,6]. Therefore, it is of global public health concern to be able to control mosquitoes effectively[7]. Most mosquito control programs aim to control the larvae and pupae with larvicides and pupicides because

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adulticides may work well only for a temporary period[8-10].

Larviciding and pupiciding are common methods for reducing mosquito population and preventing dengue and chikungunya diseases. Larvicidal activity is very important in vector management because larvae that are in the growth stage are the easiest to kill. In particular, larvae control usually depends on extended application of organophosphates or other growth regulators such as diflubenzuron and methoprene[11]. Temephos is one of an organophosphate registered and produced commercially that has been extensively used for controlling *Ae. aegypti* larvae[12].

Today, synthetic chemical insecticides used for controlling mosquito vectors are being seriously questioned because of the irreversible damages they cause to the ecosystem and the various patterns of their mosquito resistance. In recent years, it has been suggested that plant essential oils (EOs) and their constituents can be good alternative larvicidal and pupicidal agents for mosquito control, mainly because their bioactive chemicals usually cause only inconsequential side effects on other organisms and the agricultural environment[12,13]. EOs from Apiaceae and Zingiberaceae plants have been reported to have potent repellent, larvicidal, pupicidal and adulticidal activities against *Culex pipiens*, *Culex quinquefasciatus*, *Ae. aegypti*, and *Anopheles stephensi*[14-18].

In the present study, larvicidal and pupicidal activities of EOs from three Apiaceae species, namely, *Anethum graveolens* L. (*An. graveolens*), *Foeniculum vulgare* Mill. (*F. vulgare*), and *Pimpinella anisum* L. (*P. anisum*) as well as from a Zingiberaceae species, *Alpinia galanga* (L.) Willd (*Al. galanga*), were examined against *Ae. aegypti* (Linn.).

2. Materials and methods

2.1. Plant materials

Plant materials from fresh rhizomes of *Al. galanga*, dried fruits of *An. graveolens*, dried fruits of *F. vulgare*, and dried fruits of *P. anisum* were investigated in this study. The plants were identified positively by a herbal taxonomist at the Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. The rhizomes and fruits were cut into small pieces and distilled with water to obtain the EOs[10]. The plant materials were added with water at a ratio of 1:2 (plant:water) and placed in a distillation column connected to a round-bottomed distillation flask[10]. The flask was heated to about 100 ℃ and the distillation process began. It was stopped after 6 h. The EOs were dried with anhydrous sodium sulfate[10] and kept in a refrigerator at 4 ℃ until further use. Each EO was diluted to 1%, 5% and 10% in ethyl alcohol and kept in an airtight bottle at 4 ℃ for later uses[15].

2.2. Mosquito rearing

A number of *Ae. aegypti* (L.) were raised by the Department of Plant Production Technology, Faculty of Agricultural Technology, KMITL, Bangkok. The laboratory colony was kept under the following conditions: (29.5±2.0) ℃ with (75.5±2.0) relative humidity, and a photoperiod of 12-h light and 12-h dark (12L:12D). Eggs were hatched in plastic boxes (18 cm × 28 cm × 10 cm in size), each containing 1 500 mL of tap water. The larvae were fed with fish food pellets (HIPRO®) until pupation occurred. Pupae were collected and transferred to an insect cage (30 cm × 30 cm × 30 cm) and adult mosquitoes were provided with 5% glucose on cotton wool. On day 5, blood meals were given to the female adults following an artificial membrane feeding method. For egg collection, after the females were fed with blood meals for 2-3 d and ready to spawn, moist filter papers were placed on the surface of the water in a cup where they could lay eggs on. For 7 d, the eggs were kept wet and then put on a pan to hatch. The early 4th instar larvae and pupal stages of *Ae. aegypti* were tested in this experiment.

2.3. Larvicidal and pupicidal bioassay

Larvicidal activity and pupicidal activity were each determined according to a test dipping assay by Soonwera and Phasomkusolsil[10]. For each experimental treatment, 1 mL of a plant essential oil solution was added to 99 mL of distilled water in a 200 mL glass cup and shook lightly to ensure homogeneity. Ten *Ae. aegypti* in an immature stage (early fourth instar larvae or pupae stage) were put into the glass cups containing 100 mL of EOs in prepared water mentioned above. For ten larvae susceptibility test, all larvae were exposed to EOs until pupation, and mortality was observed for 24 h[10]. For ten pupae susceptibility test, the pupae were exposed to EOs until some were grown into adults, and mortality was observed for 72 h. Thirty replicates for each concentration of essential oil were performed. For comparison, a commercial formulation of temephos was used as a positive control and ethyl alcohol served as a negative control. During the period of the experiment, the larvae were offered no food[10]. They were considered dead if at the end of a 24-h period, they did not swim or move even after getting prodded by a rod. The dead and moribund larvae that showed sluggishness or abnormal movement were recorded after 24 h. Also, the pupae were recorded at 72 h and considered dead if they did not swim or move even after getting prodded by a rod[10,19].

2.4. Morphological aberrations observed

A stereomicroscope was used to determine and categorize the morphological aberrations of the dead *Ae. aegypti*, and a method described by Soonwera and Phasomkusolsil was used to categorize dead specimens[10].

Normal larvae (NL): This group represented the larvae that died after reaching the pre-pupal stage of development.

Deformed larvae (DL): This group represented the larvae that died abnormally. Dorsal splitting (arrow) of thoracic cuticle was observed in dying and dead larvae.

Pre-pupae and pupa that did not completely shed off its exoskeleton (PP): This group represented the larvae that died before they came out of their exoskeleton. Some specimens died when their heads were still enclosed in their exoskeleton.

White pupa (WP): This group represented the pupae that came out

of their larval exoskeleton completely. The white cuticle made it known as "albino".

Deformed pupae (DP): This group represented the pupae that died abnormally. In some cases, the dead pupa had an appearance of a tiny elephant and was designated "elephantoid".

Dead normal brown pupae (BP): pupae in this group were brown and normal in appearance.

Adults attached to pupal case (PA): This group represented the adults that died when they were emerging from their exoskeleton; For example, their tarsi, legs, wing and abdomen were still enclosed in their exoskeleton.

Normal adult (NA): This group represented the adults that emerged completely from their exoskeleton with normal appearance.

2.5. Oviposition deterrent assay

The oviposition deterrent activity was conducted in a laboratory using the method of Reegan *et al*[20] and a dual-choice oviposition bioassay was performed on gravid females of *Ae. aegypti*. Fifteen gravid females (5 days old) of *Ae. aegypti* were introduced into an insect cage (30 cm × 30 cm × 30 cm) under room conditions of (29.5±2.0) ℃, (75.5 ±2.0) relative humidity and 12L:12D. The adults were provided with 5% glucose solution which was available at all time. Two 200-mL plastic cups for oviposition were filled with 99 mL distilled water, one for untreated cup and the other for treated cup. One milliliter of 1%, 5% and 10% of a plant essential oil solution and temephos was added to one cup to make up the preparation for a treatment, while the untreated cup was added with 1 mL ethyl alcohol. A support for oviposition was provided by placing a piece of filter paper (Whatman® No.1) on the inner surface of each plastic cup so that the lower half of it was submerged in the treated solution or untreated solution in order for the whole paper to get moistened while the upper half of it was above the solution where the mosquitoes would lay their eggs on. The untreated and treated cups were placed at alternate diagonally opposite locations for each replicate so as to nullify any effect of their locations on oviposition. After 3 d, the number of eggs laid in the treated and untreated cups were counted under a stereomicroscope[21].

2.6. Statistical analysis

The LT_{50} and LC_{50} values were calculated using probit analysis [10]. The mortality rates that were the results of using different EOs at different concentrations were statistically analyzed by Duncan's multiple range test to compare their different efficacies.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae/pupae}}{\text{Number of larvae/pupae introduced}} \times 100$$

The oviposition activity index (OAI) was calculated using a formula used by Tikar *et al*[22]:

$$\text{OAI} = \frac{N_{treated} - N_{untreated}}{N_{treated} + N_{untreated}}$$

Where $N_{treated}$ was the number of eggs laid in the treated cups and $N_{untreated}$ was the number of eggs laid in the untreated cups. OAI was in the range of -1 and +1. Negative OAI values indicated that more eggs were laid in the untreated cup than in the treated cup and the treated solutions were a deterrent, whereas positive OAI values indicate that more eggs were laid in the treated cup than in the untreated cup, and that the treated solutions were attractive. Treatments of each concentration of EOs were replicated in six different cages. For oviposition deterrent assay, the percent effective repellency (ER) at each concentration was calculated by the following formula:

$$ER(\%) = (N_{untreated} - N_{treated})/N_{untreated} \times 100$$

Where $N_{untreated}$ was the number of eggs found in the untreated, and $N_{treated}$ was the number of eggs found in the treated.

The mean numbers of eggs deposited in the treated and untreated cups were statistically analyzed by a paired *t*-test and they were analyzed by a *t*-test and one-way analysis of variance with SPSS software (version 23.0).

3. Results

3.1. Larvicidal and pupicidal activity

The outcomes of the larvicidal bioassay on the early fourth instars of *Ae. aegypti* treated with the four EOs and the statistical data of mortality, LT_{50} and LC_{50} were shown in Table 1. All EOs at 10% concentration showed more toxicity than those at 1% and 5% concentrations. After 24 hours of exposure, it was found that *An. graveolens* EO at 1% concentration produced the mortality rate against *Ae. aegypti* at 91%, while the oil achieved 100% mortality at 5% and 10% concentrations. The result showed that LT_{50} decreased with increased concentration. Both 5% and 10% concentrations of *An. graveolens* exhibited LT_{50} values of 0.8 against *Ae. aegypti* larvae. Among the four EOs tested, the oil from *An. graveolens* exhibited the strongest larvicidal effect with the lowest lethal concentration, LC_{50} value of -0.3% (Y=109.8×X+90.71, χ^2=77.2), followed by the essential oil from *F. vulgare* with LC_{50} of 0.5% (Y=95.9×X+91.89, χ^2=155.9), *P. anisum* with LC_{50} of 0.6% (Y=362.3×X+69.34, χ^2=173.0) and *Al. galanga* with LC_{50} of 7.8% (Y=931.9×X+11.63, χ^2=289.0). On the other hand, temephos at 1% concentration (positive control) showed 94.3% mortality at 24 h with LT_{50} value of 255.7 h. Larvicidal activity of four EOs showed a positive relationship between mortality rates and exposure periods which were significant (Figure 1). The results of the pupicidal activity against *Ae. aegypti* pupae were shown in Table 2. *An. graveolens* oil at 10% concentration showed more toxicity than the oil at 1% but the same toxicity to the oil at 5%. At 1% concentration, no EOs produced any larvae mortality during the observation period. As for the results for 5% concentration, *An. graveolens* oil produced the highest mortality against *Ae. aegypti* pupae with 100% mortality at 72 h and LT_{50} value of 10.3 h, followed by *F. vulgare* with 94.0% mortality at 72 h and LT_{50} of 14.6 h. At 10% concentration, *An. graveolens* showed the highest pupicidal activity against *Ae. aegypti* pupae with 100% mortality at 72 h, LT_{50} of 6.7 h and LC_{50} of 2.9% (Y=1 066×X+9.836, χ^2=0.02), followed by the essential oil from *F. vulgare* with 99.7% mortality, LT_{50} of 7.5 h and LC_{50} of 3.5% (Y=1 062×X+7.769, χ^2=63.9), *P. anisum* with 98.3% mortality and LC_{50} of 3.84% (Y=1 052×X+7.67, χ^2=106.0) and

Al. galanga with 92.0% mortality and LC_{50} of 6.3% (Y=1 028×X-12.71, χ^2=317.0). Pupicidal activity of four EOs showed a positive relationship between mortality rates and exposure periods which were significant (Figure 2). The low value of LC_{50} for *An. graveolens* oil demonstrated its good larvicidal and pupicidal activity against *Ae. aegypti*. In contrast, temephos (positive control) showed only 4.8% mortality at 72 h with LT_{50} of 98.7 h. Not surprisingly, ethyl alcohol, the negative control, did not produce any mortality of pupae during the observation period. Therefore, cultivated in the negative control, all larvae were active and exhibited normal movement. Conversely, cultivated in the treatments, larvae were observed to have restless movements. After 1 h of treatment, all treated larvae started to have tremor and convulsion, and dead larvae started to settle to the bottom of the cup.

Table 1
Effect of herbal EOs at three concentrations (1%, 5% and 10%) against 4th instar larvae of *Ae. aegypti* at 24 h.

EOs	Concentration	Mortality±SD (%)	LT_{50} (h) (95% CL)
Al. galanga	1%	13.3±15.6	2 415.2 (2 032.5-3 054.7)
	5%	72.0±32.3	706.5 (616.9-818.9)
	10%	98.7±4.3	103.2 (2.4-2 148.2)
An. graveolens	1%	91.0±20.9	355.0 (2 514.9-511.2)
	5%	100.0±0.0	0.8 (0.6-0.9)
	10%	100.0±0.0	0.8 (0.6-0.9)
F. vulgare	1%	89.7±15.6	470.6 (396.9-565.6)
	5%	100.0±0.0	3.7 (2.6-4.7)
	10%	100.0±0.0	3.4 (2.3-3.4)
P. anisum	1%	66.0±36.6	805.4 (708.4-928.4)
	5%	100.0±0.0	1.0 (0.9-1.2)
	10%	100.0±0.0	0.8 (0.7-0.9)
Temephos	-	94.3±9.7	255.7 (20.3-2 162.0)

LT_{50} = 50% lethal time, 95% CL= 95% confidence limit.
NA: not available.

Table 2
Effect of herbal EOs at three concentrations (1%, 5% and 10%) against pupae of *Ae. aegypti* at 72 h.

EOs	Concentration	Mortality±SD (%)	LT_{50} (h) (95% CL)
Al. galanga	1%	0	NA
	5%	32.4±34.8	74.3 (66.8-84.3)
	10%	92.0±15.6	18.2 (14.3-23.3)
An. graveolens	1%	0	NA
	5%	100.0±0.0	10.3 (-)
	10%	100.0±0.0	6.7 (6.2-7.6)
F. vulgare	1%	0	NA
	5%	94.0±11.0	14.6 (9.75-21.1)
	10%	99.7±2.5	7.5 (-)
P. anisum	1%	0	NA
	5%	93.0±14.5	14.9 (8.4-25.9)
	10%	98.3±5.1	7.1 (-)
Temephos	-	4.8±10.2	98.7 (86.1-206.1)

LT_{50} = 50% lethal time, 95% CL= 95% confidence limit.
NA: not available.

3.2. Morphological aberrations

The mortality and morphological aberrations of larvae of *Ae. aegypti* were observed after 24 h of exposure to 1%, 5% and 10% concentrations of EOs which were shown in Table 3. At these concentrations, all EOs caused morphological aberrations at the time of death of the larvae. The results showed the morphological aberrational changes of *Ae. aegypti* larvae from NL to DL and deformed PP. The death of larvae at the highest mortality was usually as NL. One percent concentration of *Al. galanga*, *An. graveolens*, *F. vulgare* and *P. anisum* oils caused no morphological change (NL) at 11.6%, 84.0%, 47.7% and 56.0% NL mortality rate (Figure 3A). They caused some changes at the time of death at 1.0%, 5.3%, 39.0%, and 6.0% DL mortality rate (Figure 3B-3D). The absence of underlying epithelium in the dead larvae from EO treatment might indicate that lectin larvicidal activity was probably due to damage in the *Ae. aegypti* midgut. The deformations were such as damaged anal papillae, distorted body, darken body and shrunken cuticle. They also caused abnormal PP with deformed cephalothorax and posterior abdominal segment at 0.7%, 1.6%, 3.0% and 4.0% PP mortality rate (Figure 3E). At the concentrations of 5% and 10%, all EOs caused the greatest NL mortality rate.

All of the EOs caused some morphological aberration in the specimens during pupation after 72 hours of exposure as shown in Table 4. The main characteristic of death from EOs was DP: some abnormal pupae died with enlarged cephalothorax and wing pads were not appressed to the body; their head and body also turned black (Figure 3G-3I). Ten percent concentration of *Al. galanga*, *An. graveolens*, *F. vulgare* and *P. anisum* caused major changes almost found at the time of death at 83.0%, 75.3%, 99.0% and 97.7% DP mortality rate, respectively. However, some pupae were found dead as normal BP, their cephalothorax and abdomen had normal brown color (Figure 3F). Ten percent concentration of *Al. galanga*, *An. graveolens* and *P. anisum* oils caused no morphological change (BP) at 5.7%, 24.7% and 0.3% BP mortality rate. Some adults died while they were emerging (PA). Their tarsi, legs, wings, and abdomen were still attached to the pupal exoskeleton (Figure 3J-3K). They also caused partially emerged, tarsi-deformed adult (PA) at 3.3% (*Al. galanga*), 0.7% (*F. vulgare*) and 0.3% (*P. anisum*) PA mortality rate.

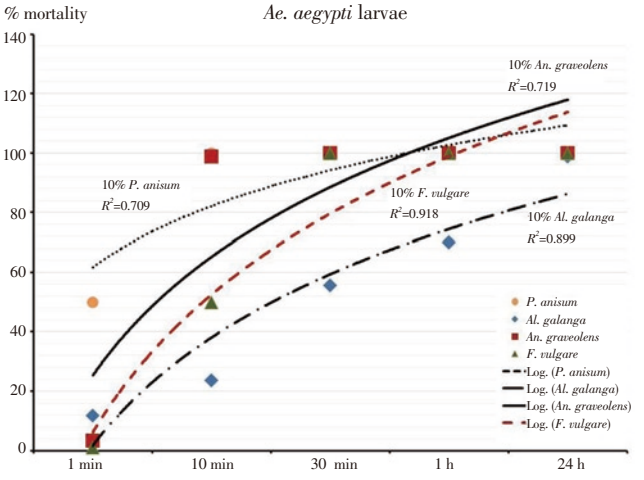

Figure 1. Relationship of mortality rate and exposure periods of larvicidal activity of essential oils against *Ae. aegypti* larvae expressed as regression.

Table 3

The effect of four EOs on morphology and mortality of *Ae. aegypti* larvae.

Instar	EOs	Concentration	Deformity at death (%)			Total motality (%)	Stage (%) (NA)
			NL	DL	PP		
Larval instar	*Al. galanga*	1%	11.6	1.0	0.7	13.3	86.7
		5%	72.0	–	–	72.0	21.3
		10%	97.4	1.3	–	98.7	20.0
	An. graveolens	1%	84.0	5.3	1.6	91.0	11.3
		5%	100.0	–	–	100.0	0
		10%	100.0	–	–	100.0	0
	F. vulgare	1%	47.7	39.0	3.0	89.7	9.0
		5%	100.0	–	–	100.0	0
		10%	100.0	–	–	100.0	0
	P. anisum	1%	56.0	6.0	4.0	66.0	34.0
		5%	100.0	–	–	100.0	0
		10%	100.0	–	–	100.0	0

NL= normal larva, DL= deformed larva, PP= pre-pupa and pupa not coming completely out of the larval exoskeleton, NA= Normal adult.

Table 4

The effect of four EOs on morphology and mortality of *Ae. aegypti* pupae.

Instar	EOs	Concentration	Deformity at death (%)			Total motality (%)	Stage (%) (NA)
			DP	BP	PA		
Pupal stage	*Al. galanga*	1%	–	–	–	–	100.0
		5%	13.7	12.3	8.3	34.3	65.0
		10%	83.0	5.7	3.3	92.0	8.0
	An. graveolens	1%	–	–	–	–	100.0
		5%	57.3	40.7	2.0	100.0	2.0
		10%	75.3	24.7	–	100.0	0.0
	F. vulgare	1%	–	–	–	–	100.0
		5%	82.0	7.6	1.7	94.0	8.7
		10%	99.0	–	0.7	99.7	0.3
	P. anisum	1%	–	–	–	–	100.0
		5%	56.0	37.33	0.67	93.0	5.3
		10%	97.7	0.3	0.3	98.3	1.7

DP =deformed pupa, BP =dead normal brown pupa, PA =adult attached to the pupal case, NA= Normal adult.

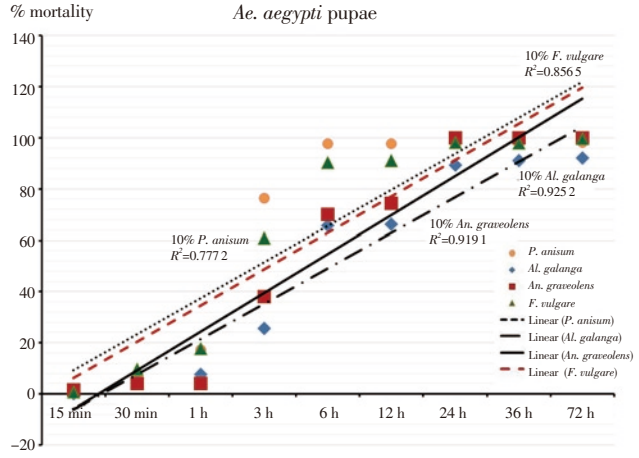

Figure 2. Relationship of mortality rate and exposure periods of pupicidal activity of essential oils against *Ae. aegypti* pupae expressed as linear regression.

3.3. Oviposition deterrent activity assay

The resulted obtained from the oviposition deterrent assay of four EOs at all three concentrations against *Ae. aegypti* were shown in Table 5. The results showed that all concentrations of all EOs were able to reduce the number of deposited eggs by gravid *Ae. aegypti* compared to the number of eggs from the gravid females treated with the ethyl alcohol. All EOs at three concentrations tested were observed to repel mosquitoes from oviposition and repellency of four EOs increased with the increase of concentration. The range of the mean number of eggs laid in the cups with the four EOs at three different concentrations 1%, 5% and 10% were 6.8-162.4. In addition, there was also a marked difference in the amount of the eggs laid. *An. graveolens* exhibited the most effective repellency activity against gravid female mosquitoes. The mean number of laid eggs in the cups with 1%, 5% and 10% of *An. graveolens* oil were 145.6, 22.6 and 6.8 eggs per cup, respectively, while the untreated cups gave a mean number of 392.0, 385.4 and 355.8 eggs per cup. A paired *t*-test confirmed that these results were significantly different ($P<0.05$). The percentage of ER caused by *An. graveolens* against oviposition were 62.9%, 94.1% and 98.1% for 1%, 5% and 10% concentrations, respectively. The range of OAI of *An. graveolens* at three concentrations was from -0.5 to -1.0. The results showed that gravid females *Ae. aegypti* preferred to lay eggs in the untreated cups rather than in the treated cups, thus it was demonstrated *An. graveolens* oil had potential to repel mosquito females for laying eggs. The present results indicated that the oviposition deterrent activity depended on concentrations as 10% *An. graveolens* oil

exhibited the strongest deterrent effect. On the other hand, temephos provided a mean number of 249.8 eggs laid per cup, while the untreated cups gave a mean number of 319.8 eggs per cup. The OAI value of temephos was -0.1; there was no significant difference in the number of eggs laid in the treated and untreated cups in this case. Therefore, temephos showed a lowest oviposition deterrent activity against *Ae. aegypti* females than those of plant EOs.

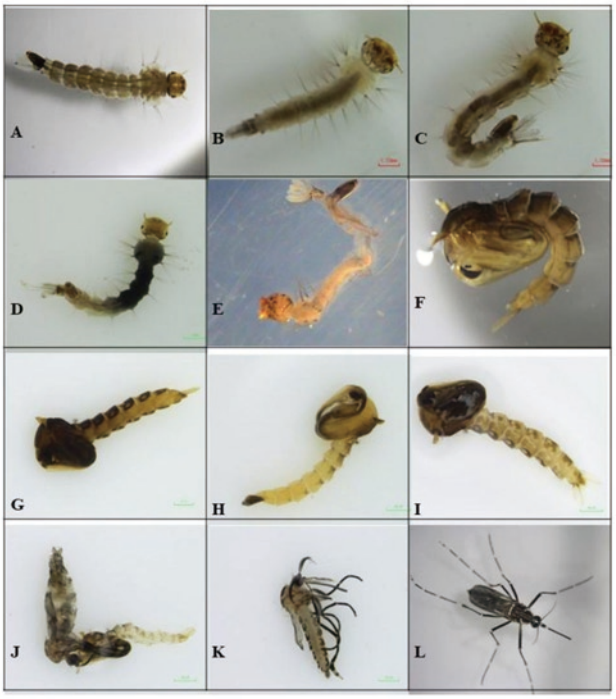

Figure 3. Morphological aberration of larvae *Ae. aegypti* after treatment with essential oils.

A: normal larvae of *Ae. aegypti* (NL); B-D: deformed larvae at death at the larval stage (DL); E: the abdomen of the pre-pupa did not come completely out of the larval exoskeleton (PP); F: a dead brown pupa (BP); G-I: deformed pupa (DP); J-K: a partially emerged, tarsi-deformed adult (PA); L: normal adult (NA).

4. Discussion

EOs derived from plants have a good potential for controlling mosquitoes in their larval and pupal stages. In the present study, 10% *An. graveolens* oil recorded the highest larvicidal and pupicidal activities of 100% mortality rate against *Ae. aegypti* immature stages.

Anethum graveolens L., commonly known as dill, is a medicinal plant with tiny yellow flowers that belongs to the plant family Apiaceae[23]. Leaves, stems and fruits of dill are widely used in various applications in the food industry, especially for their unique taste and spicy aroma[24]. Extract of *An. graveolens* obtained from the seeds have antibacterial, antispasmodic, antioxidant, antimicrobial properties[25]. The EO of *An. graveolens* exhibited a larvicidal activity among many biological activities. EO of bulk dill (pure, not in a formulation) at different concentrations (10-100 ppm) was evaluated against *Anopheles stephensi*[26]. Meanwhile, larvicidal activity was observed at the concentration of 20 ppm and increased with increasing concentration of *An. graveolens*. Lethal concentrations at 50% and 90% of *An. graveolens* EO were found to be 38.8 and 65 ppm, respectively, against the 3rd and 4th instar larvae of *Anopheles stephensi*[27]. The EO of *An. graveolens* at a concentration of 0.1 mg/mL has also shown a strong larvicidal activity against Asian tiger mosquito, *Aedes albopictus* (90% mortality)[28]. In another report, *An. graveolens* EO has also shown an effect against *Culex pipiens* adult (LC_{50} =0.495) and larvae (LC_{50} =16.996)[29]. Moreover, *An. graveolens* EO is also toxic larvicides to other insect pests. *An. graveolens* seed essential oil was found from continuous exposure and fumigant toxicity bioassays to be toxic to *Periplanata americana* L., *Musca domestica* L. and *Tribolium castaneum*. The mortality against *Periplanata americana* ranged from 25% to 100% during the first 3 h in a contact toxicity bioassay and during the first 12 h in a fumigant toxicity bioassay. In case of *Musca domestica* L., mortality ranged from 33.3% to 70.0% during the first 3 h and from 58.3% to 100.0% during the first 24 h for *Tribolium castaneum*[23]. In a previous report, the EO of *An. graveolens* was assessed for insecticidal activity against *Callosobruchus maculates* L. adults through a fumigant bioassay with LC_{50} value at 12.75 μ/L air[30]. In another study,

Table 5

The effect of four EOs on oviposition deterrent activities against *Ae. aegypti*.

EOs	Concentration (%)	Mean number of eggs ± SD		OAI	ER (%)	*P* value	Number of eggs laid per female for treatment
		Treated	Untreated				
Al. galanga	1	162.4±151.3*	629.4±344.1	-0.6	74.2	0.019	10.82
	5	59.2±41.6*	361.4±45.6	-0.7	83.6	0.032	3.94
	10	34.6±14.4*	484.0±344.1	-0.9	92.9	0.042	2.31
An. graveolens	1	145.6±59.1*	392.0±200.3	-0.5	62.9	0.040	9.71
	5	22.6±19.8*	385.4±81.6	-0.9	94.1	0.000	1.51
	10	6.8±3.5*	355.8±204.6	-1.0	100	0.019	0.45
F. vulgare	1	86.8±55.9	276.4±156.9	-0.5	68.6	0.094	5.76
	5	75.2±35.8*	308.2±161.9	-0.6	75.6	0.020	5.01
	10	13.8±7.1*	394.2±120.9	-0.9	96.5	0.002	0.92
P. anisum	1	160.8±91.5*	419.6±220.8	-0.5	61.7	0.036	10.72
	5	87.8±93.3*	519.6±211.2	-0.7	83.1	0.002	5.85
	10	69.6±88.8*	611.0±172.5	-0.8	88.6	0.001	4.64
Temephos	-	249.8±108.5	319.8±92.4	-0.1	21.0	0.046	16.65

*The significant differences between the untreated and treated group were determined by paired *t* test ($P<0.05$).

the toxicity of *An. graveolens* (leaves) plant extract at 5 and 10 mg/mL concentrations against 2-day-old (first instar) and 6-day-old (third instar) larvae of *Spodoptera litura* was investigated[31]. Many researchers have determined the chemical composition of essential oil of the fruits and seeds of *An. graveolens* which was extracted by steam distillation and hydrodistillation[32,33]. The constituents were found to be carvone, limonene, α -phellandrene, dichloromethane, α -terpinene, *p*-cymene, α - and β -pinene, γ -terpinene, cumin aldehyde, neral, trans-anethole, thymol, carvacrol, myristicin, apiol, and carotol constituents. However, the EO extracted by steam distillation contained higher amounts of limonene and carvone than the oil extracted by hydrodistillation. From the literature, carvacrol, α -pinene, and β -pinene were found to inhibit the activity of *Aedes albopictus* acetylcholinesterase with LC_{50} values of 0.057, 0.062, and 0.190 mg/mL, respectively[28]. The insecticidal property of *An. graveolens* (that contains 59% phellandrene, its most abundant compound) has been evaluated against larvae of the 3rd and early 4th instars of *Culex pipiens* with LC_{50} value of 52.74 mg/L[34]. Rocha *et al*.[35] reported morphological changes in the anal papillae of *Ae. aegypti* larvae after they were in contact with some EOs and the major chemical constituents of *An. graveolens*: (+)-limonene and (-)-limonene. After contact with these two compounds, an accumulation of dark pigmentation was observed all over the chest and at the base of the anal papillae. Structural damages to the larvae exposed to (-)-limonene include destruction of the gut and extrusion of hemolymphatic content *etc*, while damages to the larvae exposed to (+)-limonene were such as a darkening at the base of the anal papillae extended to the apex region. The present results confirm the previously reported results, revealing similar morphological changes such as changes in head and abdomen pigmentation, distorted body, darkened body and anal papillae and shrunk cuticle. The larvicidal activity of EOs may be according to diverse mechanisms. Mortality may occur at different development stages. Owing to contact effect, mode of action of EOs may act on digestive or neurological enzymes and interact with the insect's integument. Several studies have reported tremors and paralysis of larvae in their assays as well as dying larvae staying at the bottom of the containers[26,36]. In another study, it was found that several EOs blocked the effects on chemosensory receptors at the mouth parts stimulated by glucose and inositol[37]. The EOs and their constituents disrupted the endocrinological balance of the insects. They induced neurotoxicity via various mechanisms hence disrupting the normal process of morphogenesis. The damages on the muscles caused by the oils might affect the larvae's movement for respiration or feeding and the adults' development and flying ability[38].

Using for modifying the oviposition behavior of mosquitoes, oviposition deterrents and attractants play an important role in mosquito control programs. Oviposition site selected by gravid females is a critical factor that determines the proliferation and population density of the species as well as its dispersion in different geographical areas[39,40]. As female mosquitoes approach an oviposition site, they use a site-specific olfactory cue as a short-range signal for determining its quality. Volatile chemical emanated from an oviposition site is sensed and evaluated by the olfactory receptors located on the antennae, palps, labrum, and tarsi[41,42]. It has been observed that gravid females of many species of mosquitoes preferred an oviposition site over some others. This preference may be due to the presence of oviposition pheromones or oviposition attractants or repellents at the site[15,43]. In this study, *An. graveolens* oil exhibited the highest oviposition deterrent activity against female *Ae. aegypti*. The strongest activity was produced by the highest concentration of *An. graveolens* oil tested. It might produce the maximum effective repellency against oviposition by acting as a chemical signal that was detected by the sensory receptors on the antenna of the mosquitoes[44]. Warikoo *et al*.[45] have reported that pure *An. graveolens* oil deterred oviposition completely and boasted 75% effective repellency. The EO derived from dried fruits of *An. graveolens* exhibited a repellent activity against the adults of *Ae. aegypti*[46]. *An. graveolens* oil's insecticidal, oviposition deterrent, egg hatching and developmental inhibitory activities were determined against pulse beetle *Callosobruchus chinensis*[47]. Another study showed that *An. graveolens* oil reduced *Tribolium castaneum*'s oviposition potential and lengthened its developmental period in comparison with the control group. The oviposition potential of *Tribolium castaneum* decreased significantly when it was fumigated with *An. graveolens* oil[48].

The ability of gravid mosquitoes to perceive the presence of organic acids and hence detect unsuitable oviposition sites might have been acquired through evolutionary adaptation. This ability helps mosquitoes to avoid ovipositioning in unsuitable breeding sites that contain harmful toxic compounds[43]. EOs affect mosquito's nervous system; it can affect adult mosquitoes' ability to find the right host to feed on or to find the right oviposition site[38] by possible interference with nerve impulse transmission to the brain, hence changing the mosquito' response to internal and external stimuli.

In Thailand, *An. graveolens* is a cultivated crop in the Northeastern region. Its aerial part (dill weed) is a popular seasoning agent[33]. *An. graveolens* fruits in Thailand have already been used as aromatic plants and spices for food preservation and in medicine, alternative medicine and natural therapy for a long time. In this study, *An. graveolens* oil showed strong larvicidal, pupicidal and oviposition deterrent effects, thus, the good potential of *An. graveolens* oil for controlling *Ae. aegypti* has been verified. *An. graveolens* oil can be used in places where mosquitoes usually breed for controlling larvae and pupae population and for preventing egg-laying by female *Ae. aegypti* adults. If the oviposition activity of *Ae. aegypti* is inhibited, its life cycle will be disrupted and its population growth will be reduced.

In conclusion, our results clearly show that *An. graveolens* oil can be applied as larvicide, pupicide and oviposition deterrent for controlling *Ae. aegypti* population, and warrant further studies for field application. We believe that this study has demonstrated the usefulness of *An. graveolens* insecticidal properties against *Ae. aegypti*. *An. graveolens* has a full potential to be used as an inexpensive, safe and efficient larvicide on its own as well as a supplement to other larvicides.

Conflict of interest statement

The authors confirm that they have no other conflicts of interest regarding the content of this article.

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