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## Larvicidal, pupicidal and oviposition deterrent activities of essential oils from Umbelliferae plants against house fly *Musca domestica*

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### ABSTRACT

**Objective:** To evaluate the toxicity against house fly *Musca domestica* L. (*M. domestica*)-larvicidal, pupicidal, and oviposition deterrent activities of essential oils (EOs) from five plants: *Anethum graveolens* L. (*A. graveolens*), *Centratherum anthelminticum* L. (*C. anthelminticum*), *Foeniculum vulgare* Mill. (*F. vulgare*), *Pimpinella anisum* L. (*P. anisum*), and *Trachyspermum ammi* L. (*T. ammi*) Sprague. **Methods:** Dipping, topical and dual-choice assays were performed by using each EO at three concentrations (1%, 5% and 10%) as well as cypermethrin (positive control) and ethyl alcohol (negative control) in order to determine their larvicidal, pupicidal and oviposition deterrent activities against *M. domestica* mortality rate and morphogenic abnormality of larvae and pupae were recorded after 3 and 10 days of incubation. Oviposition deterrent activity was recorded for 5 consecutive days from the beginning of the assay. **Results:** All EOs at the highest concentration (10%) were highly effective in oviposition deterrent with 100% effective repellency and an oviposition activity index of -1.00. Ten percent of *F. vulgare* oil exhibited the highest efficacy against the larvae with 89.6% mortality rate at 3 days and an LC<sub>50</sub> value of 5.4%. Ten percent of *A. graveolens* and *T. ammi* oils showed the highest efficacy against the pupae with 100% mortality and an LC<sub>50</sub> value ranging from 1.81%-2.24%. **Conclusions:** These results indicate that *F. vulgare*, *A. graveolens* and *T. ammi* EOs are effective larvicidal, pupicidal and oviposition deterrent agents against house fly *M. domestica*.

## 1. Introduction

*Musca domestica* L. (*M. domestica*), also known as house fly, is one of the most common medical and veterinary insect pest in Thailand as well as throughout Southeast Asia and some other parts of the world[1,2]. It can cause serious diseases to humans and domestic animals, mechanically vectoring more than 100 human and domestic animal pathogenic organisms such as bacteria, virus, worm, parasite and protozoa and causing diseases such as food poisoning, diarrhea, cholera, bacillary dysentery, typhoid, paratyphoid, tuberculosis, salmonellosis, shigellosis, anthrax and myiasis in

humans and animals[3,4]. Generally, chemical control has been the first option for house fly control, especially during an epidemic of these diseases during the summer season (usually from March to May). Organophosphate, carbamate and pyrethroid chemical insecticides had previously been effective for house fly control. Unfortunately, house flies have developed resistance to them. Moreover, they are also highly toxic to humans, domestic animals, non-target organisms and the environment. To make matters worse, they are persistent, non-biodegradable and biomagnified through

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the food chain[5–7]. Therefore, house fly control by botanical insecticides should be a better alternative to using synthetic chemical insecticides. Botanical insecticides are ecofriendly, safe for the environment, and produce virtually no toxic effects to humans, domestic animals or non-target organisms[3]. Botanical insecticides have a long history. The first documented successful botanical insecticide was pyrethrum that has been used for delousing ever since 400 B.C. The stories of this and other more modern botanical insecticides are reported in detail in a review paper[8]. Plant essential oils (EOs) are some of the best botanical insecticides for house fly control. They have been demonstrated to be easily biodegraded in the environment and non-toxic to mammals, fish and birds[3,4,9]. Several researchers have reported that plant EOs from garlic (*Allium sativum*), galangal (*Alpinia galanga*), ylang-ylang (*Canaga odorata*), star anise (*Illicium verum*), cinnamon (*Cinnamomum verum*), clove (*Syzygium aromaticum*), lemongrass (*Cymbopogon citratus*), peppermint (*Mentha piperita*), lavender (*Lavandula angustifolia*), calamodin (*Citrus madurensis*), sweet orange (*Citrus sinensis*), eucalyptus (*Eucalyptus globulus*), ginger (*Zigiber officinale*) and phlai (*Zigiber cassumunar*) showed high toxicity to larvae, pupae and adults of house fly[10,11]. However, the number of published papers related to the oviposition deterrent activity of EOs and morphogenic abnormality of larvae and pupae of house fly is limited. Umbelliferae plant EOs, especially EOs from *Anethum graveolens* (*A. graveolens*), *Centratherum anthelminticum* (*C. anthelminticum*), *Foeniculum vulgare* (*F. vulgare*), *Pimpinella anisum* (*P. anisum*), and *Trachyspermum ammi* (*T. ammi*), have long been used to prevent and treat certain human illnesses. It has been reported that they have antimicrobial, antioxidant, antibacterial, antifungal, antiseptic, antidepressant, antispasmodic, anticancer and antineuralgic properties[12–16]. They have been shown to be highly toxic to mosquito, moth and insect pests of stored products but their larvicidal, pupicidal and oviposition deterrent activities against house fly have not been reported to date[17–19]. Data on larvicidal, pupicidal and oviposition deterrent activities against house fly are important for devising a house fly control strategy that can totally eradicate house flies at their breeding sites and sensitive areas such as the indoor of a house, hospital, primary school, nursery, nursing home and restaurant. Thus, the objective of this study was to determine the toxicity of EOs from *A. graveolens*, *C. anthelminticum*, *F. vulgare*, *P. anisum* and *T. ammi* as well as that of cypermethrin (Detroid 10) and ethyl alcohol (Siribancha) against housefly: their larvicidal, pupicidal and oviposition deterrent activities in particular.

## 2. Material and methods

### 2.1. Plant materials

The Umbelliferae plant materials were prepared from dried fruits of *A. graveolens*, *C. anthelminticum*, *F. vulgare*, *P. anisum*, and *T. ammi* purchased from Chao Krom Poe pharmacy, Bangkok, Thailand. All Umbelliferae specimens were positively identified by a plant taxonomist from King Mongkut's Institute of Technology Lardkrabang, Bangkok, Thailand. They were extracted by a water distillation method in a modified Clevenger type apparatus for 6–8 h. After the EOs were extracted by distillation, they were removed of water with anhydrous sodium sulfate and kept in a refrigerator at 4 °C until further use. All of the extracted Umbelliferae plant EOs

was diluted with ethyl alcohol to 3 concentrations: 1%, 5% and 10% and kept under laboratory conditions [(27.5±2.0) °C, (72.5±2.0)% relative humidity (RH)] before subsequent uses in assays.

### 2.2. Rearing of housefly colony

Adult house flies *M. domestica* were collected from Lardkrabang market, Lardkrabang, Bangkok, Thailand and identified by an insect taxonomist from the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Lardkrabang, Bangkok, Thailand. The 50 collected house flies were reared in a cotton mesh cage (30 cm×30 cm×30 cm) at (27.5±2.0) °C and (72.5±2.0)% RH in an entomological laboratory under a photoperiod of 12 h light followed by 12 h dark (12 L: 12 D). For 2 days, they were fed with 50 g of powdered milk, 10 g of dog biscuit, and 10% w/v glucose soaked in cotton wool. Five cotton sheets served as their oviposition substrate. The house flies laid eggs on day 3 after they were fed. One hundred house fly eggs were transferred onto 300 g of mackerel fish meat placed on a plastic tray (18 cm×30 cm×10 cm) lined with sterile coconut husks. On day 4–7 after they were laid, the eggs hatched on this medium. The 1st instar larvae fed on the mackerel fish meat and developed into 2nd instar larvae, 3rd instar larvae then pupae. Fifty house fly pupae were collected and put in a plastic box (10 cm×15 cm×7 cm) lined with sterile coconut husks. The pupae metamorphosed into adult house flies within 5 days. Two-day-old third instar larvae and pupae and 3-day-old female adults were used in bioassays.

### 2.3. Larvicidal bioassay

A dipping method as reported by Al-Ghamdi *et al*[20] was used as the larvicidal bioassay in this study. Ten third-instar larvae were selected and dipped into 10 mL of each test solution for 30 s then removed from the solution and transferred onto a filter paper in an insect box (7 cm×10 cm×5 cm in size) containing 3 g of mackerel fish meat and 2 g of moist coconut husk. Larval mortality was recorded on day 1, 2 and 3. The criterion for larval mortality was that no parts of their body were moving when prodded with a small brush. Three experimental runs were done for each kind of bioassay, and each run was performed in ten replicates. The negative and positive controls were ethyl alcohol and cypermethrin, respectively. For morphological aberration observation, dead larvae were removed from the box daily, counted and classified under a stereomicroscope. The classification criteria for larvae morphological aberrations were as follows: 1. NL=normal dead 3rd instar larvae, larvae that died with normal morphology; 2. DL=deformed larvae, larvae that died with abnormal morphology-the integument of the larvae was burned and swollen; 3. DP=deformed pupae, pupae that died with abnormal morphology-the integument of the pupae was swollen with shrunk exoskeleton; 4. DA=deformed adults, adults that died with abnormal morphology or adults that died attached to the pupal case or died partly emerged from the pupal case.

### 2.4. Pupicidal bioassay

Pupicidal bioassay was performed by using a topical application method. Ten 2-day-old pupae were placed in an insect box (7 cm×10 cm×5 cm). They were topically treated once with 10 µL of each test solution. Pupal mortality was checked by counting the

number of un-emerged pupae after 7 days of treatment. Each test was performed in 10 replicates with a simultaneous control set. The negative and positive controls were ethyl alcohol and cypermethrin. The experiment was done in three replicates.

For morphological aberration observation, dead pupae were removed daily, counted and classified under a stereomicroscope. The classification criteria for pupae morphological aberration were as follows: 1. NP=normal dead pupae, pupae that died with normal morphology; 2. DP=deformed pupae, pupae that died with abnormal morphology-the integument of the pupae was swollen with shrunk exoskeleton; 3. DA=deformed adults, adults that died with abnormal morphology-adults died attached to the pupal case or died partly emerged from the pupal case.

### 2.5. Oviposition deterrent

A double-choice method was used for oviposition deterrent bioassay. Five 3-day-old female house flies and five 3-day-old male house flies were transferred into an insect cage (30 cm×30 cm×30 cm) with a small cup (50 mL) containing powdered milk and another cup containing a ball of cotton wool soaked with 10% glucose solution. The flies fed on the food in these two cups for 3 days. Then, the cups were replaced with another two cups for the flies to oviposition (100 mL). Each cup held a sheet of cotton pad (5.0 cm×5.0 cm×0.5 cm) and was filled with 10 mL of 10% w/v milk solution. One of these latter cups was added with 1 mL of a plant EO (at 1%, 5% and 10%) that served as a treatment cup or added with 1 mL of cypermethrin (1%, 5% and 10%) that served as a positive control cup. The other one was added with 1 mL of ethyl alcohol which served as a non-treatment cup. The positions of the treatment and non-treatment cups in each insect cage were switched every day to avoid positional effect. The numbers of eggs in the treatment and non-treatment cups were counted every day under a stereomicroscope for 5 consecutive days. Each treatment was performed in five replicates and three experimental runs of the whole test were performed. The mean numbers of house fly eggs deposited in the treatment and non-treatment cups were statistically analyzed by a paired *t*-test. The oviposition activity index (OAI) and effective repellency (ER) were calculated by the following respective formulas:

$$OAI = \frac{NT-NC}{NT+NC}$$

where NT is the number of eggs laid in the treatment cup and NC is the number of eggs laid in the non-treatment cup. The interpretation of OAI is as follows: OAI=-1.0 indicates that the treatment was repellent to the flies, *i.e.*, more eggs were laid in the non-treatment cup than in the treatment cup; and OAI=+1.0 indicates that treatment was attractant to the flies, *i.e.*, more eggs were laid in the treatment cup than in the non-treatment cup.

$$ER\% = \frac{NC-NT}{NC} \times 100\%$$

where NT is the number of eggs in the treatment cup and NC is the number of eggs in the non-treatment cup. The interpretation of ER is as follows: ER=98%-100% means strongly deterrent; ER=80%-97% means fairly deterrent; ER=50%-80% means moderately deterrent; and ER<50% means not deterrent.

### 2.6. Statistical analysis

Means of larvae and pupae mortality were analyzed by standard probit analysis in order to obtain LC<sub>50</sub> and LC<sub>90</sub> for each treatment agent. The mortality data were used to compare the five EOs and the controls. Significant differences were analyzed by one-way analysis of variance and Duncan's Multiple Range Test with a software program called SPSS for windows. If the mortality caused by the negative control had exceeded 20%, the whole test would have been rejected and then repeated.

Percentage mortality was calculated by the formula below:

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

## 3. Results

### 3.1. Larvicidal bioassay

The larvicidal activity results of five Umbelliferae plant EOs against the 3rd instar larvae of house fly are summarized in Table 1. The EOs from all Umbelliferae plants at the highest tested concentration (10%) exhibited the highest mortality rates against house fly larvae; the mortality rate increased as the tested concentration increased

**Table 1**

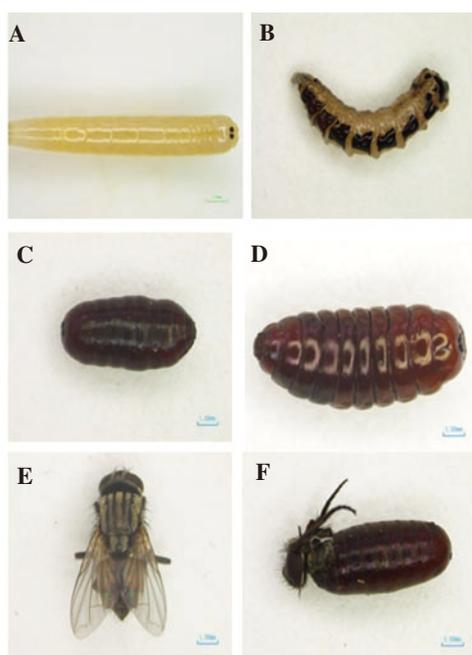
The mortality rate and LC values of *M. domestica* larvae to five Umbelliferae plants EOs, cypermethrin and ethyl alcohol at 3 days (%).

Treatment	Mortality rate (mean±SD)			LC <sub>50</sub> Lower-Upper	LC <sub>90</sub> Lower-Upper
	1%	5%	10%		
<i>A. graveolens</i> EO	13.0±10.2 <sup>b</sup>	11.7±16.6 <sup>cd#</sup>	34.3±26.6 <sup>b</sup>	15.4 (12.3, 23.2)	29.8 (22.4, 48.8)
<i>C. anthelminticum</i> EO	0.6±2.5 <sup>c#</sup>	7.0±10.5 <sup>de#</sup>	20.0±18.9 <sup>e</sup>	15.1 (13.7, 19.9)	23.2 (19.3, 30.6)
<i>F. vulgare</i> EO	16.3±9.4 <sup>b</sup>	45.7±30.2 <sup>b</sup>	89.3±14.7 <sup>a</sup>	5.4 (4.9, 6.0)	9.9 (9.1, 11.0)
<i>P. anisum</i> EO	10.3±11.8 <sup>bf#</sup>	16.0±17.3 <sup>cf#</sup>	44.7±33.9 <sup>b</sup>	11.4 (9.7, 14.4)	21.0 (17.1, 28.7)
<i>T. ammi</i> EO	2.0±5.5 <sup>c#</sup>	6.3±8.5 <sup>de#</sup>	37.3±37.8 <sup>bf</sup>	11.6 (10.4, 13.8)	17.7 (15.2, 22.6)
Cypermethrin (positive control)	100.0±0.0 <sup>a</sup>	100.0±0.0 <sup>a</sup>	100.0±0.0 <sup>a</sup>	<1.0	<1.0
Ethyl alcohol (negative control)	0.0±0.0 <sup>c</sup>	0.0±0.0 <sup>c</sup>	0.0±0.0 <sup>d</sup>	NA	NA

Mean in each column followed by the same letter are not significantly difference (*P*>0.05, by one-way ANOVA and Duncan's multiple range test); LC<sub>50</sub>=lethal concentration 50%; NA=not available. #A low EO concentration can affect the morphology and physiology of larvae but may not strongly affect their mortality, so the SD value can be higher than the mean value.

from 1% to 5% to 10%. All EOs at 10% concentration effected mortality rates ranging from (20.0%±18.9%) to (89.3%±14.7%), and the ranges of their LC<sub>50</sub> and LC<sub>90</sub> were (5.4%-15.4%) and (9.9%-29.8%), respectively. Ten percent *F. vulgare* EO exhibited the highest larvicidal activity with a mortality rate of (89.3%±14.7%) at 3 days and an LC<sub>50</sub> value and an LC<sub>90</sub> value of 5.4% and 9.9%, respectively. The ranks of Umbelliferae plant EOs according to their effectiveness as a larvicidal agent against house fly larvae were as follows: *F. vulgare* EO (10%) > *P. anisum* EO (10%) > *T. ammi* EO (10%) > *A. graveolens* EO (10%) > *C. anthelminticum* EO (10%). Cypermethrin (positive control) at all tested concentrations was also highly toxic to house fly larvae with a mortality rate of (100%±0.0%) and an LC<sub>50</sub> value and an LC<sub>90</sub> value of <1.0%, while ethyl alcohol (negative control) did not show any larvicidal activity at all.

Mortality rate and morphological aberrations of house fly larvae are presented in Table 2. Umbelliferae plant EOs at all tested concentrations caused mortality and morphological abnormality at death of most house flies in the larval stage. The morphological aberration forms varied with EO's concentration—a higher concentration induced a larger variety of forms of morphological aberration than a lower concentration did. Ten percent *F. vulgare* EO and *T. ammi* EO caused 68.3% and 17.0% mortality rates of dead larvae with deformed morphology, respectively. Deformed larvae were identified as the larvae that died with abnormal morphology and their larval integument was burned and swollen. Deformed adults were identified as the adults that died with abnormal morphology or the adults that died attached to the pupal case or partly emerged from the pupal case. Cypermethrin at all tested concentrations effected deformed larvae at 100% mortality rate while ethyl alcohol did not cause any mortality nor any morphological aberrations of house fly larvae (Figure 1).



**Figure 1.** Morphogenetic abnormalities of *M. domestica* larvae and pupae after treatment with five Umbelliferae plants EOs

A: NL=normal dead 3rd instar larva; B: DL=deformed larva; C: DP=deformed pupa; D: NP=normal dead pupa; E: DA=deformed adult; F: NA=Normal adult.

**Table 2**

Mortality rate and morphology aberrations of *M. domestica* larvae after exposure at 3 days (%).

Treatment	Conc. (%)	Stage at mortality				Total mortality	Stage of non-mortality*
		NL	DL	DP	DA		
<i>A. graveolens</i> EO	1	-	-	-	13.00	13.00	87.0
	5	-	-	-	11.70	11.70	88.3
	10	25.0	-	-	12.00	34.30	63.3
<i>C. anthelminticum</i> EO	1	0.3	-	-	0.30	0.60	99.4
	5	5.0	-	-	2.00	7.00	93.0
	10	4.4	6.0	-	9.60	20.00	79.0
<i>F. vulgare</i> EO	1	13.0	0.3	-	3.00	16.30	83.7
	5	34.0	5.0	4.7	2.00	45.70	54.3
	10	15.7	68.3	4.3	1.00	89.30	10.7
<i>P. anisum</i> EO	1	9.0	-	-	1.30	10.30	89.7
	5	14.7	-	-	1.30	16.00	84.6
	10	36.4	-	7	1.30	44.70	55.3
<i>T. ammi</i> EO	1	-	-	-	2.00	2.00	98.0
	5	-	-	-	6.33	6.33	94.0
	10	6.3	17.0	0.0	14.00	37.30	62.7
Cypermethrin	1	-	100.0	-	-	100.00	-
	5	-	100.0	-	-	100.00	-
	10	-	100.0	-	-	100.00	-
Ethyl alcohol	-	-	-	-	-	-	100.0

NL=dead normal larva, DL=deformed larva, DP=deformed pupa, DA=deformed adult. \*normal adult.

### 3.2. Pupicidal bioassay

All pupicidal activity results for the five Umbelliferae plant EOs against house fly pupae are shown in Table 3. As their concentration increased from 1% to 5% to 10%, all Umbelliferae plant EOs were increasingly more effective. All Umbelliferae plant EOs at 10% concentration effected high mortality rates, ranging from (76.0%±16.3%) to (100.0%±0.0%) with LC<sub>50</sub> values and LC<sub>90</sub> values ranging from (0.9%-5.8%) and (4.1%-14.9%), respectively. Ten percent of EOs from *T. ammi* and *A. graveolens* showed the highest toxicity against house fly pupae with the highest mortality rate of (100.0%±0.0%) at 10 days and an LC<sub>50</sub> of 2.2% and 1.8% and an LC<sub>90</sub> of 4.1% and 4.7%, respectively. The other Umbelliferae plant EOs effected mortality rates ranging from (76.0±16.3%) to (99.0%±3.1%) with LC<sub>50</sub> values ranging from (0.9%-5.8%) and LC<sub>90</sub> values ranging from (4.6%-14.9%). The ranks of umbelliferae plant EOs according to their effectiveness as a pupicidal agent against house fly pupae were as follows: *A. graveolens* EO (10%) > *T. ammi* EO (10%) > *C. anthelminticum* EO (10%) > *P. anisum* EO (10%) > *F. vulgare* EO (10%). Cypermethrin at all tested concentrations effected mortality rates ranging from (77.6%±10.1%) to (100.0%±0.0%) with an LC<sub>50</sub> of <1.0% and an LC<sub>90</sub> of 3.2%, while alcohol did not show any pupicidal activity.

All of the Umbelliferae plant EOs at all tested concentrations effected high mortality rates and induced morphological abnormalities of house flies in their pupal stage. The results are summarized in Table 4. Most Umbelliferae plant EOs at all tested concentrations caused the highest mortality rate of normal dead pupae. Dead pupae was identified as the pupae that died with normal morphology. Ten percent of EOs from, *T. ammi*, *A. graveolens*, *C. anthelminticum*, *P. anisum* and *F. vulgare* effected 100.0%, 99.0%,

**Table 3**The mortality rate and LC<sub>50</sub> values of *M. domestica* pupae to five Umbelliferae plants EOs, cypermethrin and ethyl alcohol at 10 day (%).

Treatment	Mortality rate (mean±SD)			LC <sub>50</sub> (Lower-Upper)	LC <sub>90</sub> (Lower-Upper)
	1%	5%	10%		
<i>A. graveolens</i> EO	37.7±17.5 <sup>b</sup>	92.0±12.7 <sup>a</sup>	100.0±0.0 <sup>a</sup>	1.8 (1.49, 2.11)	4.7 (4.2, 5.2)
<i>C. anthelminticum</i> EO	65.0±90.7 <sup>##</sup>	95.7±7.2 <sup>a</sup>	99.0±3.1 <sup>a</sup>	0.9 (-0.31, 1.71)	4.6 (3.7, 6.1)
<i>F. vulgare</i> EO	29.7±16.3 <sup>b</sup>	36.3±19.7 <sup>c</sup>	76.0±16.3 <sup>c</sup>	5.8 (5.03, 6.66)	14.9 (13.1, 17.8)
<i>P. anisum</i> EO	26.4±17.0 <sup>b</sup>	72.4±21.6 <sup>b</sup>	92.0±11.3 <sup>b</sup>	3.5 (2.87, 4.05)	8.7 (7.8, 9.8)
<i>T. ammi</i> EO	19.0±11.0 <sup>b</sup>	97.0±6.7 <sup>a</sup>	100±0.0 <sup>a</sup>	2.2 (2.03, 2.45)	4.1 (3.8, 4.4)
Cypermethrin (positive control)	77.6±10.1 <sup>a</sup>	94.7±8.1 <sup>a</sup>	100±0.0 <sup>a</sup>	<1 (-3.80, -1.12)	3.2 (2.7, 3.9)
Ethyl alcohol (negative control)	0 <sup>c</sup>	0 <sup>d</sup>	0 <sup>d</sup>	NA	NA
CV	101.6	18.6	9.4	-	-

Mean in each column followed by the same letter are not significantly difference ( $P>0.05$ , by one-way ANOVA and Duncan's multiple range test). LC<sub>50</sub>= lethal concentration 50%. NA=not available. # A low EO concentration can affect the morphology and physiology of pupae but may not strongly affect their mortality, so the SD value can be higher than the mean value.

99.0%, 90.0% and 73.3% mortality rates of normal dead pupae and 10% cypermethrin effected 100% mortality rates of normal dead pupae, but ethyl alcohol did not kill any pupae and did not cause any morphological abnormality of forms. All Umbelliferae plant EOs at 1%, 5% and 10% concentrations caused pupae deaths as deformed pupae with mortality rates ranging from 0.7%-3.0%, 0.7-9.0% and 0.3%-2.0%, respectively. Deformed pupae was pupae that died with abnormal morphology or their pupal integument was swollen with shrunk exoskeleton at the time of death. All Umbelliferae plant EOs at 1%, 5% and 10% concentrations caused mortality of deformed adults at rates ranging from 0.3%-2.0%, 0.3-3.4% and 0.7%-1.3%, respectively (Table 4, Figure 1).

**Table 4**Mortality rate and morphology aberrations of *M. domestica* pupae after exposure at 10 days (%).

Treatment	Conc. (%)	Stage at death			Total mortality	Stage of non- mortality*
		NP	DP	DA		
<i>A. graveolens</i> EO	1	34.7	1.0	2.0	37.7	62.3
	5	81.0	9.0	2.0	92.0	8.0
	10	99.0	0.3	0.7	100.0	-
<i>C. anthelminticum</i> EO	1	63.0	2.0	-	65.0	35.0
	5	92.4	-	3.3	95.7	4.3
	10	99.0	-	-	99.0	1.0
<i>F. vulgare</i> EO	1	29.0	0.7	-	29.7	70.3
	5	34.9	0.7	0.7	36.3	62.0
	10	73.7	2.0	0.3	76.0	24.0
<i>P. anisum</i> EO	1	25.4	0.7	0.3	26.4	73.6
	5	68.7	3.7	-	72.4	28.3
	10	90.0	1.3	0.7	92.0	8.0
<i>T. ammi</i> EO	1	16.0	3.0	-	19.0	81.0
	5	91.7	5.0	0.3	97.0	1.3
	10	100.0	-	-	100.0	-
	1	5.7	2.0	14.7	77.6	7.3
	5	88.3	2.7	3.7	94.7	1.3
Cypermethrin	10	100.0	-	-	100.0	-
Ethyl alcohol	-	-	-	-	100.0	100.0

NP=dead normal pupa, DP=deformed pupa, DA=deformed adult; \*normal adult.

### 3.3. Oviposition deterrent

The oviposition deterrent activities of five Umbelliferae plant EOs

and cypermethrin against female adults of house flies are presented in Table 5. All Umbelliferae plant EOs at a high concentration (10%) exhibited high percentage ER against female house flies, and as their concentration increased from 1% to 5% to 10%, the effectiveness of all Umbelliferae plant EOs correspondingly increased. Ten percent of all Umbelliferae plant EOs showed the highest oviposition deterrent activity with the highest percentage effective repellency at 100% and an OAI of -1.0. Moreover, 5% EOs from *A. graveolens*, *C. anthelminticum* and *T. ammi* also showed the highest oviposition deterrent activity with the highest effective repellency at 100% and an OAI of -1.0. One percent of all Umbelliferae plant EOs showed a fair to moderate oviposition deterrent activity with 42.40% to 87.78% ER and an OAI ranging from 0.21 to -0.78. At the same time, all concentrations of cypermethrin exhibited the highest oviposition deterrent activity with 100% ER and -1.0 OAI. The ranks of Umbelliferae plant EOs according to their effectiveness as a oviposition deterrent agent against female house flies are as follows: *T. ammi* EO (10%)>*C. anthelminticum* EO (10%)>*A. graveolens* EO (10%)>*F. vulgare* EO (10%)>*P. anisum* EO (10%).

## 4. Discussion

The following are reported pharmacological activities of *F. vulgare* EO: antimicrobial, antiviral, anti-inflammatory, antiallergic, hepatoprotective, antixiolytic and anti-stress[21], while those of *A. graveolens* EO are as follows: antiallergic, antimicrobial, antiviral, anti-inflammatory, antixiolytic and anti-stress[15], and the reported pharmacological activities of *T. ammi* EO are as follows: analgesic, antinociceptive, anthelmintic, antifilarial, antiinflammatory, antiplatelet, antimicrobial, and antiviral[22].

The test results showed that the three EOs from *F. vulgare*, *A. graveolens* and *T. ammi* exhibited strong activities against the larvae and pupae of house fly as well as a very strong oviposition deterrent activity. They have a good potential for being developed into a powerful agent for controlling all stages of house fly in their life cycle.

*F. vulgare* is an important medicinal plant belonging to the Umbelliferae family. Its common name is fennel. It originated from

Table 5

The oviposition deterrent activities of the five Umbelliferae plant EOs in three concentrations (1%, 5% and 10%) against *M. domestica* females.

Treatment	Conc. (%)	Number of eggs (mean±SD)		OAI	ER (%)	Susceptibility	Number of eggs laid per female for treatment	P-value
		Treatment cup	Non-treatment cup					
<i>A. graveolens</i> EO	1	98.7±78.9*	520±200.3	-0.68	81.03	FD	19.70	0.020
	5	9.7±80.0*#	526.7±75.2	-0.96	98.16	SD	1.90	0.050
	10	0.0±0.0*	305.3±228.5	-1.00	100.00	SD	0.00	0.010
<i>C. anthelminticum</i> EO	1	44.7±43.5	275.0±226.8	-0.72	83.76	FD	8.94	0.190
	5	0.0±0.0*	492.7±150.6	-1.00	100.00	SD	0.00	0.030
	10	0.0±0.0*	390.7±98.1	-1.00	100.00	SD	0.00	0.020
<i>F. vulgare</i> EO	1	223.3±94.3	388.0±111.6	-0.27	42.40	N	64.40	0.300
	5	71.7±16.7*	447.7±232.5	-0.72	83.90	FD	14.30	0.240
	10	0.0±0.0*	756.0±193.3	-1.00	100.00	SD	0.00	0.020
<i>P. anisum</i> EO	1	329.0±131.5	214.7±146.2	0.21	-	N	65.80	0.540
	5	104.3±153.0*#	545.3±190.2	-0.68	80.87	FD	20.90	0.140
	10	0.0±0.0*	530.7±388.1	-1.00	100.00	SD	0.00	0.000
<i>T. ammi</i> EO	1	57.3±11.8*	469.0±85.7	-0.78	87.78	FD	11.46	0.020
	5	0.0±0.0*	436.3±133.7	-1.00	100.00	SD	0.00	0.030
	10	0.0±0.0*	427.7±73.4	-1.00	100.00	SD	0.00	0.010
Cypermethrin	1	0.0±0.0*	154.3±56.2	-1.00	100.00	SD	0.00	0.020
	5	0.0±0.0*	276.0±62.9	-1.00	100.00	SD	0.00	0.038
	10	-	81.67±65.9	-1.00	100.00	SD	0.00	0.019

\*The significant differences between the treatment and non-treatment group were determined by paired *t* test ( $P < 0.05$ ). OAI=oviposition activity index; ER%=percent effective repellency. ER=98%-100%, strongly deterrent (SD); ER=80%-97% were indicated as fairly deterrent (FD); ER=50%-80% were indicated as moderately deterrent (MD); ER < 50% were indicated as no-effect (N). #A low EO concentration showed a lower repellency activity against female housefly than a high EO concentration, so the SD value can be higher than the mean value.

southern Mediterranean region and now grows wild throughout Asia, North America and Europe[23]. Fennel is well known in Thailand as ‘Thian klaep’[23,24]. It has been used for a long time in traditional Thai medicine as an aromatic ingredient. By itself, it is carminative, expectorant, tonic, digestive and diuretic[25]. The fruits and seeds of *F. vulgare* are also used as an aromatic food additive in ice cream, alcoholic beverages as well as meat and fish dishes[23,26]. Ten percent *F. vulgare* EO was noted to be the most toxic to house fly larvae. Most larvae that died because of this EO died as dead normal larvae. In the same vein, papers published in 2018 reported that 5% and 10% *F. vulgare* EO showed a high larvicidal activity against *Aedes aegypti* (*Ae. aegypti*) mosquito with 100% mortality at 24 h and an  $LT_{50}$  value of 3.4 h[27]. Most larvae that died because of this EO died as dead normal larvae[27]. *F. vulgare* EO also showed a strong adulticidal activity against *M. domestica* with an  $LD_{50}$  value ranging from 82-89  $\mu$ g/adult[28]. In addition, *F. vulgare* EO was highly effective against the 4th instar larvae of *Ae. aegypti*, *Anopheles dirus*, *Anopheles stephensi* (*An. stephensi*) and *Culex pipiens* (*Cx. pipiens*) mosquitoes[29–31] and exhibited a high repellent activity against *Ae. aegypti* females[32,33]. This EO has also been shown to be highly toxic to three important insect pests of stored-product (*Sitophilus oryzae*, *Callosobruchus chinensis* and *Lasioderma serricorne*)[33]. The major chemical components of *F. vulgare* EO are monoterpenes, trans-anethole, estragole, fenchone, methyl chavicol, eugenol, limonene, *p*-anisaldehyde,  $\alpha$ -phellandrene,  $\gamma$ -terpineol,  $\alpha$ -pinene, *p*-cymene and 1,8-cineole[23,26,34]. These monoterpenes have been shown to have an insecticidal activity against house fly[35]. Moreover, *p*-cymene, 1,8-cineole and limonene as fumigants

were more toxic to adult house flies than all of the other chemical components mentioned above[35]. Fenchone from *F. vulgare* EO was found to inhibit the activity of cholinergic system of insects[34]. The 1,8-cineole and  $\gamma$ -terpineol monoterpenoids from this EO were found to inhibit photoreception of *Cx. pipiens* compound eyes[17]. In addition, trans-anethole and limonene were shown to be highly toxic to insect neuron cells[36]. In the present investigation, house fly larvae exposed to *F. vulgare* EO died with abnormalities such as becoming pigmented, having dark colored cuticle, shrunken or its body becoming twisted. This EO might penetrate into the cuticle of house fly larvae, inhibiting the activities of their nervous system and interfering with their metamorphosis. More importantly, *F. vulgare* EO shows very low toxicity to mammals; its  $LD_{50}$  value against rats is 3 120 mg/kg; it also has a very low persistence effect on the environment[34,37]. Therefore, *F. vulgare* EO has a good potential as a natural larvicide for controlling house fly larvae.

In this respect, field trials at real house fly breeding sites are needed to be carried out to further explore its practical use. Against house fly pupae, 10% *A. graveolens* EO showed the highest toxicity; it caused the highest mortality rate of dead normal pupae. *A. graveolens* is a medicinal and aromatic plant. It belongs to the Umbelliferae family. Its common name is dill[13]. Dill is well known in Thailand as ‘Thian ta takkataen’ and used in traditional Thai medicine for a long time as carminative, expectorant, tonic, digestive and diuretic[24]. The leaves, stem and fruits of *A. graveolens* are well known to be aromatic and used as a common spice in Thai cuisine[25,38]. In the same vein, 5% and 10% of *A. graveolens* EO has been reported to show high toxicity against the pupae of *Ae. aegypti* mosquito with 100% mortality at 72

h and an  $LT_{50}$  value ranging from 6.7 h to 10.3 h. It also caused the highest morphological abnormality in dead pupae[27]. In addition, EO from *A. graveolens* seed exhibited a high fumigant toxicity against adult house flies with a mortality rate ranging from 33.3% to 70.0% at 3 h[13]. In another report, EO from *A. graveolens* fruits showed high toxicity against *Aedes albopitus*, *Anopheles stephensi* and *Anopheles gambiae* larvae and adults of other insect pests such as *Cx. pipiens*, *Periplaneta americana*, *Tribolium castaneum* and *Callosobruchus maculatus*[32,33,39,40]. The main chemical components of *A. graveolens* EO are carvone (57.3%), limonene (33.2%),  $\alpha$ -pinene,  $\beta$ -pinene,  $\alpha$ -phellandrene, *p*-cymene, carvacrol and  $\alpha$ -terpinene[15,22,38]. Carvone, *p*-cymene and carvacrol from this EO showed high toxicity against adult house flies[23]. Carvacrol,  $\alpha$ -pinene,  $\beta$ -pinene and phellandrene were highly toxic to the larvae of *Ae. aegypti* and *Cx. pipiens*[13]; they have been reported to be toxic to the neuron cells of many insects[32]. In the present investigation, house fly pupae exposed to *A. graveolens* EO died with abnormalities such as contorted body and shrunk or malformed body. This EO might penetrate into the cuticle of house fly pupae and exert its toxicity to the neurons of their nervous system, inhibiting the normal process of morphogenesis and metamorphosis[27].

In this study, *A. graveolens* EO exhibited a strong pupicidal activity, thus it has a good potential as an agent for controlling house fly pupae. *A. graveolens* EO can be used in places where house flies usually breed in order to control pupae population. House fly life cycle will be disrupted, and its population growth will be reduced.

Plant EOs disrupts an important female mosquitoes' behavior: ovipositioning behavior or search attempt for a suitable ovipositioning area[41]. In this study, *T. ammi* EO at all tested concentrations exhibited the highest repellency and oviposition deterrent activity against female house flies. *T. ammi* EO also showed high ovicidal and oviposition deterrent activities against *Callosobruchus chinensis* eggs and females[42]. This EO exhibited high toxicity against the larvae of *An. stephensi* and adults of *Tribolium castaneum* and German cockroach, *Blattella germanica*[19,43–45]. The common name of *T. ammi* is ajwain or ajowan. It originated from India and became widely distributed in Afganistan, Iran, Pakistan and Europe[42]. This plant has been used as medicine and food. It is well known in Thailand as 'Thain yaowa phani' and has been used in traditional Thai medicine for a long time as anti-emetic, blood tonic, expectorant and carminative[46]. The main chemical constituents of the EO from *T. ammi* fruit are thymol (63.4%),  $\alpha$ -terpinene (16.9%) and *p*-cymene (19.0%)[44]. In a previous report, thymol and *p*-cymene from plant EOs as fumigants were found to be highly toxic against house fly adults[35]. In addition, *p*-cymene and  $\gamma$ -terpinene as fumigants showed high toxicity against both females and males of *Blattella germanica*. In the same vein, thymol was demonstrated to have strong contact toxicity against both sexes of *Blattella germanica*[43]. Moreover, thymol from plant EOs such as from *Thymus vulgaris* and *T. ammi* also showed high toxicity against insects: a high inhibitory activity against two neurotransmitters

(gamma-aminobutyric acid and octopamine) of insect's nervous system[36]. The following explanation for the results from this study is offered: *T. ammi* EO and its chemical constituents might penetrate into the tarsal and labial cuticles of female house flies and inhibit the activities of their neuron cells and ovipositor. This EO also showed the highest effective repellency against female house flies, hence we proposed it as a good natural oviposition deterrent agent for controlling female house flies that needs some field trials at real house fly breeding sites to validate whether it can be a practical natural house-fly oviposition deterrent agent for full control of house fly population or not.

The results from this study indicate that EOs from *F. vulgare*, *A. graveolens* and *T. ammi* would be highly toxic to house flies at all stages of their life cycle. Their larvicidal, pupicidal and oviposition deterrent efficacies were much higher than ethyl alcohol (the negative control) but slightly lower than cypermethrin (the positive control) in larvicidal activity and equivalent to cypermethrin in pupicidal and oviposition deterrent efficacies.

Cypermethrin is a synthetic pyrethroid. It is a broad-spectrum insecticide in wide scale use globally for controlling agricultural, medical and veterinary insect pests such as insect pests of plants and vegetables, insect pest of stored products, mosquito vectors, house fly and horse fly[47]. Unfortunately, cypermethrin has been indicated to be moderately hazardous to humans by World Health Organization[48]. To make matters worse, cypermethrin is highly toxic to fishes, beneficial insects and other predatorial and parasitic insects such as honey bee, digger bee, leaf cutting bee, trichogramma, braconid and ensign wasp[47]. Its harmful effects to human and animal health are such as cytogenesis effect on human lymphocytes, induction of apoptosis in human neuroblastoma cells causing neuroblastoma in children, and oxidative stress effect on female reproductive system of rats and rabbits[47,49,50].

Our results indicate that *F. vulgare*, *A. graveolens* and *T. ammi* EOs would be effective against house fly at all stages of its life cycle. The EOs have a good potential to be developed into an effective, all-around, natural larvicidal, pupicidal and oviposition deterrent agent for completely controlling house fly population, especially at their breeding and ovipositioning sites. Most importantly, they are much safer to humans and biodegrades in the environment much faster than cypermethrin. Finally, the findings from this study can be used for further development of these EOs into a practical agent that can truly disrupt all stages of house fly life cycle and effectively reduce the whole of its population, hence they can help reduce all serious diseases vectored by house flies to human beings.

### Conflict of interest statement

The authors confirm that they have no conflicts of interest with any parties whatsoever regarding the content of this article.

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