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## Larvicidal and Oviposition deterrent activities of essential oils against house fly (*Musca domestica* L.; Diptera: Muscidae)

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House fly (*Musca domestica* L.) is one of the most important insect pests of household and livestock, their has been developed resistance to chemical insecticides. In fact, green pesticides derived from plant essential oils are an excellent alternative to synthetic pesticides. In the present study, the Thai essential oils derived from ylang-ylang (*Cananga odorata* Lamk), citronella grass (*Cymbopogon nardus* (L.) Rendle) and clove (*Syzygium aromaticum* L.) were evaluated for their larvicidal and oviposition deterrent activity against house fly and compared them with chemical insecticide (cypermethrin 10.0% w/w, kumakai 10<sup>®</sup>). The highest larvicidal activity and oviposition deterrent activity was shown by *S. aromaticum* oil with LC<sub>50</sub> values of 9.83% and 100% effective repellency and -1.0 OAI at 1.65µl/cm<sup>2</sup>. Moreover, *S. aromaticum* oil also exhibited the higher larvicidal and oviposition deterrent activity than cypermethrin. However, toxicity assay indicates the order of LC<sub>50</sub> values and percentage of effective repellency against house fly in three essential oils as *S. aromaticum* oil > *C. odorata* oil > *C. nardus* oil. These results clearly revealed that *S. aromaticum* oil served as green pesticides to control house fly population and safe for human and environmental friendly.

**Key words:** herbal essential oils, larvicide, oviposition deterrent, *Musca domestica*

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## Introduction

House fly, *Musca domestica* L. (Diptera: Muscidae) is an important medical and veterinary insect pests causing a serious diseases to human and livestock by vectoring more than 100 human and animal pathogenic organisms such as protozoa cysts, helminth parasites, enteropathogenic bacteria and enterovirus (Tarelli *et al.*, 2009; Hana, 2013). In Thailand, Maipanich *et al.* (2010) reported the eleven kinds of helminth eggs and larvae were found on house flies in fifty one tourist areas in twenty two province of Thailand, including Bangkok, such as *Ascaris lumbricoides*, *Trichuris trichiura*, *Enterobius vermicularis*, *Taenia* spp., *Spirometra* sp., *Opisthorchis viverrini*, *Echinostoma* sp. and *Eurytrema* sp. In fact, control of house fly commonly depended on chemical pesticides, because of their speedy action and easy application. Unfortunately, the resistance of house fly to chemical pesticides have increased (Khan *et al.*, 2013). Moreover, chemical pesticides also have toxic side effect to humans, animals and environment (Sinthusiri and Soonwera, 2013). However, the alternative strategies for house fly control are need. Therefore, the environmental friendly and biodegradable natural pesticides from plants origin have been receiving attention as an alternative green pesticide for controlling insect pests (Phasomkusolsil and Soonwera, 2012). The essential oils from plants or herbs are documented for exhibition of acute toxicity, anti-feeding and oviposition deterrents against a wide variety of insect pest, including house fly (Isman, 2006; Koul *et al.*, 2008). Moreover, plant essential oils considered for controlling house fly because of their selectivity, high toxicity for insect, target specificity, minimal environmental effects and safety to humans (Tarelli *et al.*, 2009; Kumar *et al.*, 2013). Besides, many researchers have reported the bioefficacy of essential oils from *Cymbopogon citratus*, *Cymbopogon nardus*, *Cymbopogon winterianus*, *Citrus sinensis* and *Pogostemon cablin* were evaluated for their insecticidal activity against larvae, pupae and adults of house fly (Palacios, 2009; Pavela, 2008, Kumar *et al.*, 2011; Kumar *et al.*, 2012; Morey and Khandagle, 2012; Sinthusiri and Soonwera, 2010). Sinthusiri and Soonwera (2013) also reported that the insecticidal effects of twenty essential oils derived from herbs against adult of house fly, the most effective were shown by *C. citratus* oil, *M. piperita* oil and *L. angustifolia* oil, showing 100% mortality at 24 h. and LT<sub>50</sub> (median lethal time) values of 2.22, 2.62 and 3.26 min., respectively. However, *C. odorata* oil exhibited high potential of oviposition deterrent and ovicidal action against three mosquito species (*Aedes aegypti*, *Anopheles dirus* and *Culex quinquefasciatus* (Phasomkusolsil and Soonwera, 2012).

Therefore, the aim of this study was to investigate the larvicidal effect and oviposition deterrent activity of three essential oils from Thai herbs (*Cananga odorata*, *Cymbopogon nardus* and *Syzygium aromaticum*) against house fly (*M. domestica*).

## **Materials and Methods**

### ***House fly***

Adult house flies were collected through sweep net method from markets in Ladkrabang, Bangkok, Thailand and identified by Entomologist of Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. The ten of adult house flies (5 males : 5 females) were reared in screen cages (30x30x30 cm), 10% w/v glucose + 10% w/v milk and 300 g. of mackerel fishes as a food source and oviposition site. The one hundred larvae fed on 300 g of mackerel fishes, the 3<sup>rd</sup> instar larvae and female adult aged 4-5 days old were used for this study.

### ***Plant materials and essential oils***

Various parts of three Thai plant species (*Cananga odorata* (Flower), *Cymbopogon citratus* (Stem) and *Syzygium aromaticum* (Flower)) were collected from Nakhonratchasima province, Thailand. All plant was identified by Plant Taxonomist of Plant Production Technology Section, Faculty of Agricultural Technology, KMITL. The various plant parts were extracted for essential oil by water distillation. Each essential oil was prepared as 1%, 5% and 10% solutions in ethyl alcohol and stored at 4°C before testing.

### ***Chemical insecticide***

Cypermethrin 10% w/v (Kumakai 10<sup>®</sup>), a common chemical insecticide for insect pest control in Thailand was purchased from MD Industry Co., Ltd., 22 Phahonyothin Rd., Wongnoi, Prakhonsri ayutthaya province, Thailand, used as standard.

### ***Larvicidal bioassays***

The bioassay was evaluated by using dipping method (Sripongpun, 2008). The ten of 3<sup>rd</sup> instar larvae were dipped into 10 ML of each test solution for 10 sec. and transferred to a filter paper (in plastic box; 7.5x10.0x7.5 cm), containing a diet of 10 g. of mackerel fishes. Larval mortality was recorded at 1.0, 6.0, 12.0 and 24.0 h. The criteria for larval mortality was evaluated by softly touching each on with a small paint brush and if they not responding were

considered dead (Sinthusiri and Soonwera, 2010). Each test was performed in five replicates. The data were pooled and analyzed by standard probit analysis to obtain a  $LT_{50}$  (median lethal time) and  $LC_{50}$  (median lethal concentration).

### ***Oviposition deterrent bioassay***

The ten of female adult house flies aged 4-5 days old, were introduced in a screened cage where two oviposition boxes (7.5x10.0x7.5 cm) lined with cotton pad (3x10x0.25 cm) were introduced for oviposition. For two oviposition boxes, the 1<sup>st</sup> box as tested box was filled with 1 ML of 10% w/v milk solution and 1 ML of each test solution, while, the 2<sup>nd</sup> box served as control was filled with only 1 ML of 10% w/v milk solution. The position of the boxes were switched everyday to avoid the position effects. The eggs laid into each box were collected separately until no further eggs were laid for at least 48h. The egg collected were counted using a stereomicroscope. Five replicates were carried out with each test solution. The percentage of effective repellency for each essential oil was calculated using the following formula (Phasomkusolsil and Soonwera, 2012; Warikoo *et al.*, 2011).

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

Where ER = effective repellency

NC = the total number of eggs in the control solution

NT = the total number of eggs in each test solution

The oviposition experiments were expressed as mean number of eggs and oviposition activity index (OAI), which was calculated using the following formula (Phasomkusolsil and Soonwera, 2012)

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

NC = the total number of eggs in the control solution

NT = the total number of eggs in each test solution

The OAI ranges from -1.0 to + 1.0, with 0 indicating neutral response. The positive index values indicate that more eggs were deposited in the test boxes than in the control boxes, and that the test solutions were attractive, on the other side, more eggs in the control boxes than in the test boxes results in negative index values and the test solutions were deterrent.

## Results and Discussion

The larvicidal effect of three essential oils and cypermethrin against 3<sup>rd</sup> instar larvae of house fly as shown in Table 1. On the LT<sub>50</sub> values and LC<sub>50</sub> values, the results revealed that *S. aromaticum* oil exhibited highest larvicidal effect against house fly larvae with LT<sub>50</sub> values of 27.05 h. and LC<sub>50</sub> values of 9.83%, followed by *C. nardus* oil and *C. odorata* oil with LT<sub>50</sub> values of 38.99 and 52.08 h. and LC<sub>50</sub> values of 13.60 and 29.36%, respectively. While, cypermethrin showed LT<sub>50</sub> values of 31.63 h. and LC<sub>50</sub> values of 11.45%, these LT<sub>50</sub> values and LC<sub>50</sub> values exhibited fewer toxicity than *S. aromaticum* oil.

For oviposition deterrent experiments, the results as shown in Table 2. The house fly females preferred to lay eggs in control boxes than test boxes. However, all test solution at 1.65 µl/cm<sup>2</sup> showed fewer oviposition deterrent activity against house fly females than at 3.30 µl/cm<sup>2</sup>. The highest percentage of effective repellency (ER%) was shown by *S. aromaticum* oil and *C. odorata* oil with 100% in 1.65µl/cm<sup>2</sup> and 3.30 µl/cm<sup>2</sup> and -1.0 OAI (Oviposition Activity Index). For *C. nardus* oil exhibited 98.92 to 99.25% ER and -0.98 to -0.99 OAI. While, cypermethrin showed 100% ER and -1.0 OAI. Therefore, the results indicated that all herbal essential oil and cypermethrin were excellent oviposition deterrent against house fly females.

Our study has revealed that the essential oil from *S. aromaticum* showed the excellent larvicidal and oviposition deterrent activity against larvae and females of house flies and these results exhibited higher toxic than cypermethrin. Moreover, *S. aromaticum* oil also showed the high percentage of effective repellency against oviposition of *Ae. aegypti* and *Cx. quinquefasciatus* with 93.30 and 98.40% and -0.9 and -1.0 OAI, respectively (Phasomkusolsil and Soonwera, 2012). Furthermore, Trongtokit *et al.* (2005) studied the mosquito repellency of 38 essential oils and found *S. aromaticum* oil to provide the longest and most effective protection

time against three mosquito species (*Aedes* sp., *Anopheles* sp. and *Culex* sp.). Shapiro (2012) also reported that eugenol is a natural chemical found in oil of *S. aromaticum* that attracts Japanese beetles, kill insects, and is considered safe without risks when used as directed, and the US Environmental Protection Agency (USEPA) classified *S. aromaticum* oil as minimum risk pesticides. However, *S. aromaticum* oil has been studied for its antibacterial, antimicrobial and antifungal properties against cutaneous infectious manifestations and has been shown to be environmentally safe and nontoxic to humans for use in medicine, perfume and food flavoring. Moreover, *S. aromaticum* oil is commonly used in dental root canal surgery for its antimicrobial properties in Thailand (Trongtokit *et al.*, 2004).

For *C. odorata* oil showed the excellent oviposition deterrent with 100% effective repellency against oviposition of house fly females. Phasomkusolsil and Soonwera (2012) supported these results that, *C. odorata* oil also exhibited high potential for oviposition deterrent and ovicidal action against three mosquito species (*Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus*). In addition, essential oils from *C. odorata* and *Lippia alba* showed the most active repellent properties against *Tribolium castaneum* (Coleoptera) (Gallardo *et al.*, 2011). However, many researcher reported the chemical properties and therapeutic properties of *C. odorata* oil such as Burdock and Carabin (2008) reported the essential oil extracted from flower of *C. odorata* by water distillation showed physical and chemical properties and the constituents of this oil were phenols, eugenol, methyleugenol, isoeugenol, limonene, geraniol and cinnamaldehyde. These constituents have properties to act as toxins, feeding deterrents and oviposition deterrents to a wide variety of insect pests (Koul *et al.*, 2008). Moreover, *C. odorata* oil showed therapeutic properties, such as antidepressant, antiseborrhoeic, antiseptic, aphrodisiac, hypotensive, nervine and sedative (Phasomkusolsil and Soonwera, 2012).

However, *C. nardus* oil showed the moderate of larvicidal activity against house fly larvae, but this oil showed high oviposition deterrent activity against house fly females. Our results, supported by Sinthusiri and Soonwera (2010) reported that oil of *C. nardus* showed high insecticidal activity against 2<sup>nd</sup> instar larvae, pupae and adults of house flies with 100% mortality at 20.0 min., 3 days and 120 sec., respectively. Besides, *C. nardus* oil or citronella oil is approved for use on human as an insect repellent with little or no known toxicity (EPA, 2009).

In the present study it was found that three essential oils from Thai herbs showed high potential as larvicide and oviposition deterrent for house fly control. All essential oil in this study has high potential for development of new product or green product to house fly management. Finally, the green products base on herbal essential oils is considered environmentally safe and

offer safer human health alternatives to insect pest control with chemical insecticides, and could be a good option for Thai people.

## Conclusion

Thai essential oils from *S. aromaticum*, *C. odorata* and *C. nardus* exhibited high potential for development of green pesticides for house fly control.

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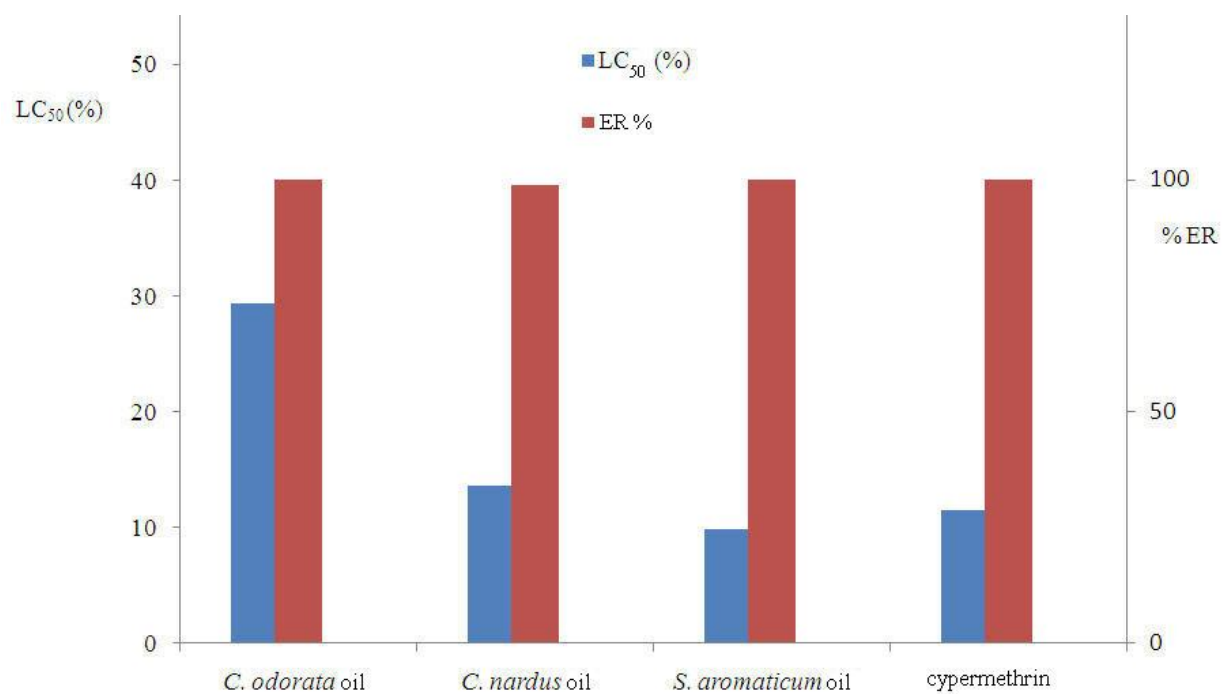
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**Figure 1.** Median lethal time (LC<sub>50</sub>) and percentage of effective repellency (ER%) of three essential oils and cypermethrin against *Musca domestica*.

**Table 1.** Larvicidal effect of three essential oils from Thai herbs and chemical insecticide against 3<sup>rd</sup> instar larvae of *Musca domestica*.

Treatments	Lethal Time (LT <sub>50</sub> ) (h.)	Lethal Concentrations (%)
<i>C. odorata</i> oil (ylang-ylang)	52.08	29.36
<i>C. nardus</i> oil (citronella grass)	38.99	13.60
<i>S. aromaticum</i> oil (clove)	27.05	9.83
cypermethrin	31.63	11.45

**Table 2.** Oviposition deterrent effect of three essential oils from Thai herbs and chemical insecticide against *Musca domestica* female.

Treatment	Concentration ( $\mu\text{l}/\text{cm}^2$ )	Effective Repellency (%)	OAI
<i>C. odorata</i> oil (ylang-ylang)	1.65 $\mu\text{l}/\text{cm}^2$	100	-1.0
	3.30 $\mu\text{l}/\text{cm}^2$	100	-1.0
<i>C. nardus</i> oil (citronella grass)	1.65 $\mu\text{l}/\text{cm}^2$	98.92	-0.98
	3.30 $\mu\text{l}/\text{cm}^2$	99.25	-0.99
<i>S. aromaticum</i> oil (clove)	1.65 $\mu\text{l}/\text{cm}^2$	100	-1.0
	3.30 $\mu\text{l}/\text{cm}^2$	100	-1.0
cypermethrin	1.65 $\mu\text{l}/\text{cm}^2$	100	-1.0
	3.30 $\mu\text{l}/\text{cm}^2$	100	-1.0