International Journal of Agricultural Technology 2022Vol. 18(1):271-280
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Adulticidal activity against housefly (*Musca domestica* L.; Muscidae: Diptera) of eucalyptol, limonene, and their combined formulation

Moungthipmalai, T. and Soonwera, M.*

Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Chalong Krung Road, Ladkrabang, Bangkok 10520, Thailand.

Moungthipmalai, T. and Soonwera, M. (2022). Adulticidal activities of eucalyptol, limonene, and their combination against housefly. (*Musca domestica* L. ; Muscidae : Diptera). International Journal of Agricultural Technology 18(1):271-280.

Abstract The insecticidal potency of eucalyptol, limonene, and a combined formulation against housefly (*Musca domestica*) was evaluated and compared to that of cypermethrin, a common synthetic insecticide. The knockdown and mortality rates were determined by a standard susceptibility assay recommended by World Health Organization. The tested concentrations of eucalyptol and limonene were 1, 5, and 10%, while the combined formulation was 5% eucalyptol + 5% limonene. The highest efficacy (100% mortality rate) was provided by the combined formulation (5% eucalyptol + 5% limonene). Both individual essential oil constituents provided a mortality rate ranging from 10.7-82.0% and a knockdown rate ranging from 8.0-81.3%. Most importantly, the 5% eucalyptol + 5% limonene combined formulation provided as high a mortality and knockdown rate as that of 10% cypermethrin. Therefore, it has a good potential as a safer and equivalently effective, natural product alternative to cypermethrin.

Keywords: Eucalyptol, Insecticidal activity, Limonene, *Musca domestica*

Introduction

Housefly (*Musca domestica* L.) is the most common species of flies. Houseflies usually habituate in tropical regions of the world but can spread to everywhere all over the world. Houseflies have membranous wings and are generally smaller than 6-10 mm. Their body is in various shades of grey with four longitudinal black stripes on the thorax and yellow stripes on both sides of the abdomen. They are well-adapted to human habitats and live through their whole life cycle in the habitats such as hospital, market, slaughterhouse, restaurant, and farm of livestock (Iqbal *et al*., 2014; Khamesipour *et al*., 2018).

Houseflies are serious public health and livestock pest because they spend most of their life and perform their reproduction process in unsanitary

* **Corresponding Author**: Soonwera, M.; **Email:** mayura.so@kmitl.ac.th, mayura.soon@gmail.com

environments such as those containing feces, carrion, and human waste, so their body parts are usually covered with viral or bacterial disease vectors. More than 100 kinds of human pathogens from those unsanitary environments get mechanically attached to some body parts of houseflies, and as they fly into human habitats, transferred those pathogens to humans. Those various kinds of pathogens to humans and livestock include bacteria, protozoa, fungi, viruses, and parasitic worms. Many of them cause deadly diseases to humans, such as avian influenza and diarrheal diseases. The production of a livestock farm may decrease because the animals are annoyed by houseflies and their normal feeding and milking behaviors may be negatively affected. An example is poultry farm. Houseflies not only cause stress to poultry, but also carry poultry pathogens that cause deadly colibacillosis and necrotic enteritis diseases. Another example is cattle farm, Thailand's Department of Livestock Development has recently reported an unprecedented spread of lumpy skin disease (LSDV) in Thailand, a long-discovered cattle disease in Africa (Kanjanapusit, 2021). LSD viruses are carried in nature by several arthropod vectors, such as stable fly (*Stomoxys calcitrans*), mosquito (*Aedes aegypti*), hard tick (*Rhipicephalus and Amblyomma* sp.), and common housefly (*Musca domestica*) (Sprygin *et al*., 2019).

Since they have been introduced, synthetic chemicals have been used to effectively control housefly populations, but they are quite harmful to human health. Common synthetic chemicals for controlling houseflies are pyrethroids (beta-cyfluthrin, deltamethrin, permethrin, transfluthrin), which can be in various dosage forms (e.g., coil, aerosol spray, and other types of fumigants). At the present time, they are not only harmful to humans but also not as effective as they were when they were introduced due to developed insect resistance over time (Freeman *et al*., 2019; Khan *et al*., 2017; Soonwera and Sittichok, 2020; Wang *et al*., 2019).

For these reasons, new agents for controlling housefly population are urgently needed. Effective and safe candidates are plant EOs and their major constituent. For example, EOs from *Mentha piperata*, *Cymbopogon citratus*, *Citrus sinensis*, *Eucalyptus globulus*, and their combinations are repellent and larvicidal against *M. domestica* and *Anopheles stephensi*. These EOs contain the following active major constituents: menthol and menthone (*M. piperata*); citral (*C. citratus*); limonene and myrcene (*C. sinensis*); and 1,8-cineol/eucalyptol (*E. globulus*) (Chauhan *et al*., 2018), which have also been reported to be effective against *M. domestica*. Moreover, some EOs and EO constituents have been combined into more potent, synergistic formulations, such as a formulation of combined EOs of 5% *C. citratus* + 5% *E. globulus* and a formulation of combined EO constituents, 5% 1,8-cineole + 5% geranial, which were highly effective against the adults of *Ae. aegypti*, *Ae. albopictus*, and *M. domestica* (Soonwera and Sittichok, 2020).

The main objective of this study was to assess the adulticidal efficacy against housefly (*M. domestica*) of eucalyptol and limonene and a combined formulation of them.

Materials and methods

Housefly rearing

A sweeping net technique was used for collecting adult houseflies from a local market in Bang Sao Thong District, Samut Prakan Province, Thailand. After the collection, they were positively identified by an entomologist at King Mongkut's Institute of Technology, Thailand. Later, 50 of the collected adult houseflies (25 males and 25 females) were reared in an insect cage ($30\times30\times30$ cm^3) under laboratory conditions of 30 ± 2 ℃ temperature and 70-80% humidity. They were fed with 10% glucose + 1% multivitamin soaked in cotton sheets. After 3-4 days of rearing, female houseflies laid eggs on a cotton sheet ($5\times6.5\times0.5$ cm^3) soaked with 10 ml of milk in a plastic cup (8.5 cm in height and 3.5 cm in diameter). After that, the eggs were placed on pieces of steamed mackerel in a plastic box ($18.5\times27\times10$ cm), which was then sealed with adhesive tape. After 12-24 hours, the eggs began to hatch into first instar larvae. The development of first instar to fourth instar took around 4 days, and then the fourth instar started to pupate. In a plastic cup in an insect cage, 100 housefly pupae were reared. When adults emerged from the pupae, they were subjected to the standard WHO susceptibility assay (WHO, 2018).

Major EO constituents

Eucalyptol and limonene were manufactured by Sigma-Aldrich and purchased by the Entomological Laboratory, Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. All formulations containing an individual EO constituent and their combination in this study are detailed in Table 1.

Other chemicals

Cypermethrin (Dethroid 10®), at 1, 5 and 10% w/v, the positive control, was manufactured by Pentacheme Co., LTD., 214-216 Charoenakhon Road, Khongsan, Bangkok 10600, Thailand), and 70% Ethyl alcohol (Siribuncha®), the negative control, was manufactured by Siribuncha Co., LTD., 50/4 Mu7 Banggruay-Sainoi Rd., Nonthaburi Province, Thailand; www.siribuncha.com).

Table 1. formulations of eucalyptol and limonene alone as well as their combination against the adults of *M. domestica*

EO constituent and their combination	formulation
eucalyptol	1% eucalyptol + 99% ethyl alcohol
eucalyptol	5% eucalyptol + 95% ethyl alcohol
eucalyptol	10% eucalyptol + 90% ethyl alcohol
limonene	1% limonene + 99% ethyl alcohol
limonene	5% limonene + 95% ethyl alcohol
limonene	10% limonene + 90% ethyl alcohol
eucalyptol + limonene	5% eucalyptol + 5% limonene + 90% ethyl alcohol

Knockdown and mortality assay

The knockdown and mortality assay on adult houseflies was the same as the assay reported by Sinthusiri and Soonwera (2013). The susceptibility assay was the standard World Health Organization (WHO, 2018) Susceptibility Test. Definitions of various susceptibility levels (WHO, 2018) are as follows: 98.0-100% mortality rate denotes susceptibility (S); 80.0-97.0% mortality rate denotes possible resistant that needs confirmation (PR); and less than 80.0% mortality rate denotes resistant (R).

The experimental design was a completely randomized design. Three replicates of each of the 11 different treatments were run. Cypermethrin at 1, 5, and 10% were the positive control, and 70% Ethyl alcohol was the negative control. In the susceptibility test, ten 2-to-3-day-old adult houseflies (5 males:5 females) were exposed to each treatment in a treatment tube (the size of 44 mm in diameter and 125 mm in length). Two milliliters of a treatment were dropped onto a filter paper (the size of 12×15 cm, Whatman No1®), and the soaked filter paper was put in the treatment tube. After 1 h of exposure, the houseflies were transferred to the non-treatment tube (containing a plain, intact filter paper). At 1, 5, 10, 15, 30, and 60 min after exposure, the knockdown rates were recorded, while the mortality rates were recorded at 24 h after exposure. The criterion for knockdown was that there was no movement of any housefly body parts when the houseflies were prodded with a soft brush within an hour, but the insects would recover afterwards, while the criterion for mortality was the same no movement of body parts but for at least 24 h after exposure. Knockdown rate (KR%) and mortality rate (MR%) were calculated by the following formula:

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

$$\text{Mortality rate (MR\%)} = [ND/NC] \times 100,$$

where NK was the total number of knocked down houseflies; ND was the total number of dead houseflies; and NC was the total number of treated adult houseflies. KT_{50} (50% knockdown time) was calculated by probit analysis. Mortality data were analyzed with Duncan's Multiple Range Test (DMRT).

In addition, we defined an Effective Knockdown Index (EKI) to efficiently indicate how much higher or lower the efficacy of a formulation was versus cypermethrin in terms of knockdown efficacy as follows,

Effective Knockdown Index (EKI) = [KEO/KC],

where KEO was the KT_{50} of each formulation of individual or combined EO constituents, and KC was the KT_{50} provided by cypermethrin at the same concentration.

Hence, EKI < 1 would indicate that the formulation of individual or combined EO constituents was better at knocking down housefly than cypermethrin at the same concentration (a KT_{50} value is a time value, hence the shorter the better); EKI > 1 would indicate the contrary; and EKI=1 would indicate equivalent toxicity.

In the same vein, we defined an Effective Mortality Index (EMI) to efficiently indicate how much higher or lower the efficacy of each EO constituent formulation and combined formulation was versus cypermethrin in terms of mortality. EMI was calculated by the formula below,

Effective Mortality Index (EMI) = [MEO/MC],

where MEO was the mortality rate of each formulation of individual EO constituent and a formulation of combined EO constituents, and MC was the mortality rate provided by cypermethrin at the same concentration.

However, unlike the meaning of EKI, EMI<1 indicated that the individual EO constituent or the combined formulation was less effective at killing housefly than cypermethrin at the same concentration; EMI>1would indicate the contrary; and EMI=1 would indicate equivalent killing efficacy.

Results

Susceptibility test

The knockdown rate, KT_{50}, and Effective Knockdown Index (EKI) of individual EO constituent and their combination against housefly (*M. domestica*) are presented in Table 2. The most effective individual EO constituent formulation was 10% eucalyptol, followed by 5% eucalyptol, 1% eucalyptol, 10% limonene, 5% limonene, and 1% limonene. However, the 5%

eucalyptol + 5% limonene combined formulation was even more effective than 10% eucalyptol with a KT_{50} of 5.5 min versus 17.8 min provided by eucalyptol alone. Moreover, the knockdown rate provided by 5% eucalyptol + 5% limonene was nearly identical to the rate provided by10% cypermethrin and higher than 5% and 1% cypermethrin, even though the combination's KT_{50} was not as short (see Figure 1). In terms of EKI, all individual and combined EO constituent formulations were less effective at knocking down houseflies than cypermethrin at the same concentration.

Table 2. Knockdown rate, KT_{50}, and Effective Knockdown Index (EKI) against houseflies (*M. domestica*) at 60 min after exposure to eucalyptol, limonene, and their combined formulation

Treatment	Knockdown rate ±SD	KT_{50} (min)	EKI
1% eucalyptol	8.0 ±19.3	113.8	2.8
5% eucalyptol	24.7 ±40.3	90.3	8.3
10% eucalyptol	81.3 ±31.6	17.8	25.4
1% limonene	3.3 ±12.2	2774.7	68.0
5% limonene	12 ±16.7	2094.2	192.1
10% limonene	16 ±11.6	1736.9	2481.3
5% eucalyptol + 5% limonene	100 ±0	5.5	7.9
1% cypermethrin	56.7 ±32.4	40.8	-
5% cypermethrin	90.7 ±12.2	10.9	-
10% cypermethrin	100 ±0	0.7	-
70% ethyl alcohol	0	na	-

KT_{50} = 50% Knockdown time;
na: not computable by Probit analysis;
EKI = Effective Knockdown Index.

The mortality rate, LC_{50}, WHO susceptibility status, and Effective Mortality Index (EMI) of individual and combined EO constituent formulations against housefly (*M. domestica*) are presented in Table 3. In terms of mortality rate, the most effective individual EO constituent formulation was 10% eucalyptol, followed by 10% limonene, 5% eucalyptol, 5% limonene, 1% eucalyptol, and 1% limonene, with a mortality rate ranging from 10.7 to 82.0%. The combined 5% eucalyptol + 5% limonene formulation was more effective than any individual EO constituent formulations, providing a mortality rate of 100%, as effective as 10% cypermethrin and more effective than 5% and 1% cypermethrin. In terms of LC_{50}, which could not be defined properly for the combined EO constituent formulation, cypermethrin provided the lowest LC_{50}, followed by eucalyptol, and limonene, with an LC_{50} of 6.0, and 13.0, respectively. In terms of WHO susceptibility status, 5% eucalyptol + 5% limonene and 10% cypermethrin had a susceptible (S) status; 10% eucalyptol and 5% cypermethrin had a Possible Resistant (PR) status; and 1% cypermethrin, 10% limonene, 5% eucalyptol, 5% limonene, 1% eucalyptol, and 1% limonene had a Resistant (R) status. In terms of EMI, 5% eucalyptol + 5% limonene was more effective at killing houseflies than cypermethrin at the same concentration. However, all individual EO constituent formulations were less effective at that than cypermethrin.

Table 3. Mortality rate, LC_{50}, WHO susceptibility status, and Effective Mortality Index (EMI) against houseflies (*M. domestica*) at 24 h after exposure of eucalyptol, limonene, and their combined formulation

Treatment	Mortality rate ±SD	LC_{50}(%)	Susceptibility	EMI
1% eucalyptol	10.7±18.7		R	0.2
5% eucalyptol	36.0±36.6	6.0	R	0.4
10% eucalyptol	82.0±27.8		PR	0.8
1% limonene	10.7±12.2		R	0.2
5% limonene	27.3±16.7	13.0	R	0.3
10% limonene	37.3±11.6		R	0.4
5% eucalyptol + 5% limonene	100±0	-	S	1
1% cypermethrin	56.7±32.4		R	-
5% cypermethrin	96.0±6.3	0.7	PR	-
10% cypermethrin	100±0		S	-

Remarks: LC_{50} = Lethal concentration that kills 50% of treated houseflies;
WHO status: S = Susceptible is defined as 98–100% mortality; PR = Possible Resistant is defined as 80.0–97.0% mortality; and R = Resistant is defined as < 80% mortality;
EMI= Effective Mortality Index was defined as the ratio of the mortality rate provided the treatment to the mortality rate provided by cypermethrin at the same concentration.

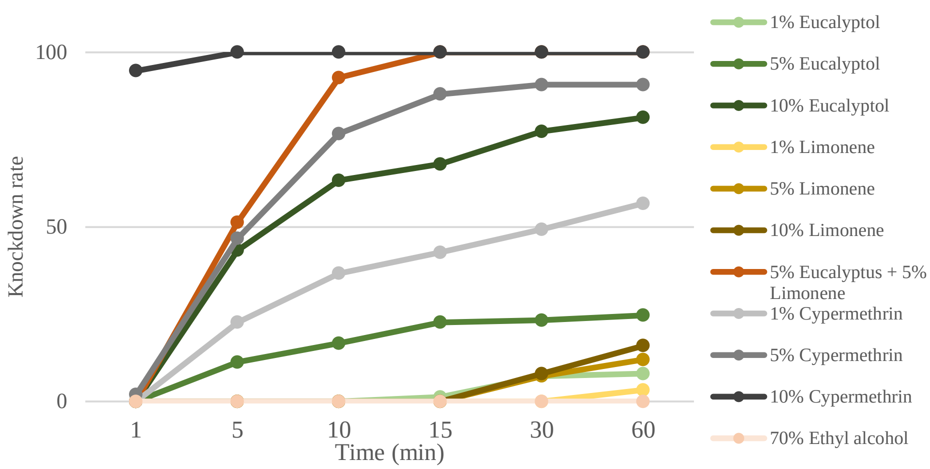

Figure 1. Linear regression between % knockdown rate and exposure time (min) of individual EO constituent formulations and a combined formulation against houseflies

Discussion

Three main topics are discussed. The first main topic is that the combined 5% eucalyptol + 5% limonene formulation provided as high a mortality rate and a knockdown rate against *M. domestica* as those of 10% cypermethrin, implying that this formulation can be as equally effective as cypermethrin if developed into a commercial insecticidal product (Chantawee and Soonwera, 2018). The potency of this formulation was expected to be high before we had conducted the study because each component of the combined formulation, eucalyptol and limonene, has already been reported to be high by several previous studies: Kumar *et al*. (2013 and 2014), Palacios *et al*. (2009), and Sukontason *et al*. (2004).

The second main topic is that the combined formulation was most likely synergistic in its insecticidal activity than individual eucalyptol or limonene alone: 100% mortality rate (combined formulation) versus 82% (10% eucalyptol) and 37.3% (10% limonene). This kind of synergy between constituents of different EOs was reported by Scalerandi *et al.* (2018) and Soonwera and Sittichok (2020).

The third and final main topic is that the mortality rates provided by eucalyptol and limonene that we observed in this study were in full agreement with the mortality rates observed by Palacios *et al*. (2009), suggesting that both of our methods and findings were valid to a certain extent. To conclude, all of our findings indicate that a combined formulation of 5% eucalyptol + 5% limonene has a good potential for replacing cypermethrin as a safer and equivalently effective natural insecticide alternative.

Acknowledgments

This work was supported by King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, 10520 Thailand, in a grant for KMITL Doctoral Scholarship (Grant No. KDS 2021/002). We also wish to express our gratitude to Mr. Pratana Kangsadal, a KMITL Proofreader, for editing the manuscript.

References

Chantawee, A. and Soonwera, M. (2018). Larvicidal, pupicidal and oviposition deterrent activities of essential oils from Umbelliferae plants against house fly *Musca domestica*. Asian Pacific Journal of Tropical Medicine, 11:621-629.

Chauhan, N., Malik, A. and Sharma, S. (2018). Repellency potential of essential oils against housefly, *Musca domestica* L. Environmental Science and Pollution Research, 25:4707-4714.

Freeman, J. C., Ross, D. H. and Scott, J. G. (2019). Insecticide resistance monitoring of house fly populations from the United States. Pesticide Biochemistry and Physiology, 158:61-68.

Iqbal, W., Malik, M. F., Sarwar, M. K., Azam, I., Iram, N. and Rashda, A. (2014). Role of housefly (*Musca domestica*, Diptera; Muscidae) as a disease vector; a review. Journal of Entomology and Zoology Studies, 2:159-163.

Kanjanapusit, S. (2021). Strict control of lumpy skin disease in cattle. Thailand's Department of Livestock Development. Retrieved from https://dld.go.th/th/index.php/th/newsflash/341-news- /23707-hotiss ue-25640407-1.

Khamesipour, F., Lankarani, K. B., Honarvar, B. and Kwenti, T. E. (2018). A systematic review of human pathogens carried by the housefly (*Musca domestica* L.). BMC Public Health, 18:1049.

Khan, H. A. A., Akram, A. and Fatima, A. (2017). Resistance to pyrethroid insecticides in house flies, *Musca domestica* L., (Diptera: Muscidae) collected from urban areas in Punjab, Pakistan. Parasitology Research, 116:3381-3385.

Kumar, P., Mishra, S., Malik, A. and Satya, S. (2013) Housefly (*Musca domestica* L.) control potential of Cymbopogon citratus Stapf. (Poales: Poaceae) essential oil and monoterpenes (citral and 1,8-cineole). Parasitology Research, 112:69-76.

Kumar, P., Mishra, S., Malik, A. and Satya, S. (2014) Biocontrol potential of essential oil monoterpenes against housefly, *Musca domestica* (Diptera: Muscidae). Ecotoxicology and Environmental Safety, 100:1-6.

Scalerandi, E., Flores, G. A., Palacio, M., Defagó, M. T., Carpinella, M. C., Valladares, G., Bertoni, A. and Palacios, S. M. (2018). Understanding synergistic toxicity of terpenes as insecticides: contribution of metabolic detoxification in *Musca domestica*. Frontiers in Plant Science, 9:1579.

Palacios, S. M., Bertoni, A., Rossi, Y., Santander, R. and Urzúa, A. (2009). Efficacy of essential oils from edible plants as insecticides against the house fly, *Musca Domestica* L. Molecules, 14: 1938-1947.

Sinthusiri, J. and Soonwera, M. (2013). Efficacy of herbal essential oils as insecticides against the housefly, *Musca domestica* L. The Southeast Asian Journal of Tropical Medicine and Public Health, 44:188-196.

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.

Sprygin, A., Pestova, Y., Wallace, D. B., Tuppurainen, E. A. and Kononov, V. (2019). Transmission of lumpy skin disease virus: a short review. Virus Research, 269:1976372.

Sukontason, K. L., Boonchu, N., Sukontason, K. and Choochote, W. (2004). Effects of eucalyptol on house fly (Diptera: Muscidae) and blow fly (Diptera: Calliphoridae). Revista do Instituto de Medicina Tropical de São Paulo, 46:97-101.

Wang, J. N., Hou, J., Wu, Y. Y., Guo, S., Liu, Q. M., Li, T. Q. and Gong, Z. Y. (2019). Resistance of house fly, *Musca domestica* L. (Diptera: Muscidae), to five insecticides in Zhejiang province, China: the situation in 2017. Canadian Journal of Infectious Diseases and Medical Microbiology, 2019:4851914-4851914.

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.

(Received: 9 May 2021, accepted: 20 December 2021)