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Adulticidal activity against houseflies (*Musca domestica* L.; Muscidae: Diptera) of combinations of *Cymbopogoncitratus* and *Eucalyptus globulus* essential oils and their major constituents

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.

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Abstract The efficacies of *Cymbopogon citratus*, *Eucalyptus globulus*, eucalyptol, geranial, and their combined formulations against houseflies (*Musca domestica*) were evaluated and compared to cypermethrin, a common synthetic insecticide. Using a standard susceptibility assay that the World Health Organization (WHO) advises, the knockdown and mortality rates were calculated. The tested concentration range of these essential oils and their constituents was between 1% to 5%. The highest efficacy (100% knockdown rate and mortality rate) were provided by a combined formulation of 2% *E. globulus* EO + 1% geranial. This formulation had a KT_{50} of 4.27 min. The two next best formulations were 2% *C. citratus* EO + 1% eucalyptol that provided a KT_{50} of 5.85 min and an individual 5% *C. citratus* EO that provided a KT_{50} of 6.69 min. Most importantly, several tested formulations provided a higher knockdown rate than 1% cypermethrin. Therefore, these formulations have a full potential as an alternative natural insecticide to replace cypermethrin.

Keywords: *Cymbopogon citratus,* Eucalyptol, *Eucalyptus globulus,* Geranial, *Musca domestica*

Introduction

Houseflies (*Musca domestica* L.) are common insects that spread throughout the world. They typically reside close to areas that are home to humans and livestock, including hospitals, restaurants, food markets, slaughterhouses, and livestock farms. Housefly is a serious public health and livestock pest. They are not only a nuisance but also a vector of more than 100 diseases. Many are deadly, such as avian influenza and diarrheal diseases (Issa, 2019; Khamesipour *et al*., 2018). Examples of affected livestock farms are sheep and

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

goat farms. Houseflies not only stress sheep and goats, but they also spread infections that give them terrible illnesses like sheeppox and goatpox.

They are caused by ORF virus (Poxviridae). ORFV in secretion of houseflies enters its hosts through the skin and usually remains confined to the epidermis, inducing formation of papules, nodules, or vesicles that develop into thick crust or heavy scab mainly on the lips, nostrils, eyes, breast, and distal part of the limbs. Accompanying high fever can cause mortality. Occasionally, this kind of infection can happen to humans. Typically, an infected person develops a single, painful nodule at the exposed areas, mostly at the hands and arms and, occasionally, the face (Raele *et al*., 2021).

Currently, houseflies have developed rapid resistance to synthetic insecticides including pyrethroids (alpha-cypermethrin, bifenthrin, deltamethrin, cyfluthrin, and cypermethrin), organophosphates (fenitrothion, malathion, diazinon, pirimiphos-methyl, and chlorpyrifos), and insect growth regulators (diflubenzuron, triflumuron, pyriproxyfen, methoxyfenozide, and cyromazine). Houseflies were cross-resistance to fourteen insecticides from different chemical classes (Hafez, 2022).

For all of these reasons, it is urgent to find a substitute for these synthetic insecticides. Natural insecticides are plant EOs and EO constituents. For example, EOs of *Achillea ligustica*, *Lippia alba*, *Cinnamomum verum*, *Pelargonium odoratissimum*, *Helichrysum italicum*, *Ocimum basilicum*, *Mentha spicata*, and *Mentha* x *piperita* cause acute toxicity against adults of *M. domestica.* The most toxic EOs were EOs from *C. verum* and *H. italicum*, followed by *P. odoratissimum*, *Mentha* x *piperita*, *O. basilicum*, and *M. spicata* (Benelli *et.al.*, 2018). Two more examples are EOs from *C. citratus* and *E. globulus* and their combinations. A formulation of combined EOs, 5% *C. citratus* + 5% *E. globulus*, and a formulation of combined EO constituents, 5% 1,8-cineole + 5% geranial, provides synergistically high knockdown and mortality rates against adults of *M. domestica* (Soonwera and Sittichok, 2020).

The primary goal was to evaluate the effectiveness of *C. citratus*, *E. globulus*, *eucalyptol*, and geranial as well as their combination formulations as adulticides against houseflies (*M. domestica*).

Materials and methods

EOs, EO constituents and their combinations

Essential oils from *C. citratus and E. globulus* were obtained from the Entomological Laboratory, Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang

(KMITL), Thailand. Analytical-grade eucalyptol and geranial were supplied by Sigma-Aldrich. All formulations are detailed in Table 1.

Table 1. Formulations of *C. citratus* EO, *E. globulus* EO, eucalyptol, geranial and their combination

EO, EO constituent and their combination	formulation
C. citratus EO	5% *C. citratus* EO in ethyl alcohol
E. globulus EO	5% *E. globulus* EO in ethyl alcohol
Eucalyptol	1% eucalyptol in ethyl alcohol
Geranial	1% geranial in ethyl alcohol
C. citratus EO + eucalyptol	2% *C. citratus* EO + 1% eucalyptol in ethyl alcohol
E. *globulus* EO + geranial	2% E. *globulus* EO + 1% geranial in ethyl alcohol

Comparative chemicals

Pentacheme Co., LTD., located at 214-216 Charoenakhon Road in Khongsan, Bangkok, Thailand, produced the positive control, 1% w/v cypermethrin (Dethriod10®). Siribuncha Co., LTD., 50/4 Mu7 Banggruay-Sainoi Rd., Nonthaburi Province, Thailand; www.siribuncha.com, produced the negative control, 70% ethyl alcohol (Siribuncha®).

Housefly rearing

Adult houseflies were collected by sweeping net technique from a local market in Ladkrabang District, Bangkok Province, Thailand. An entomologist from King Mongkut's Institute of Technology Ladkrabang, Thailand, successfully recognized them. Fifty adult houseflies were raised in a climate of 70-80% humidity and 30±2 °C temperature. They received 10% glucose and 1% multivitamin dissolved in cotton sheets as food. Those sheets were placed in a box in a rearing cage. Another box covered with moist coconut peat and steamed mackerel was also in the cage to simulate ideal conditions for egg-laying. After the adult female houseflies laid eggs, the eggs were transferred to another bigger plastic box (18.5x27x10 cm) filled with coconut peat. This box was then sealed with adhesive tape. Care was taken so that the hatched larvae would have sufficient food. After 3-4 days, third instar larvae started to pupate. One hundred pupae were transferred from the box to plastic cups, which were put in a new rearing cage. Adults were tested using the standard WHO susceptibility assay after they emerged from the pupae (WHO, 2018).

Knockdown and mortality assay

The adult housefly knockdown and mortality assays were the same assays as Soonwera and Sittichok (2020) described. The experimental design was completely randomized. A treatment tube measuring 44 mm in diameter and 125 mm in length was used to administer 2 milliliters of each treatment to ten adult houseflies that were 3 days old (5 males and 5 females). A filter paper (12 x 15 cm, Whatman No1®) was used to contain the treatment. The houseflies were moved to a non-treatment tube (which contained nothing, intact filter paper) after 1 hour of exposure. The knockdown rate was recorded at 1 h after exposure, and the mortality rate was recorded at 24 h. Each treatment was performed in three replicates. The positive control was 1% cypermethrin, and the negative control was 70% ethyl alcohol. There was no movement of any body parts, which was the requirement for knockdown and mortality. The Susceptibility Test conducted by the World Health Organization (WHO, 2018) was used to determine the susceptibility status. According to WHO's 2018 definitions, there are several vulnerability levels: Less than 80.00% mortality rate indicates resistance (R), 80.00-97.00% mortality rate indicates possible resistance (PR), and 98.00–100% mortality rate indicates susceptibility (S). Knockdown rate (KR%) and mortality rate (MR%) were calculated by the following formulas:

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

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

where NK represented the overall number of houseflies that were knocked down, ND represented the overall number of houseflies that were killed, and NT represented the overall number of treated adult houseflies. Through probit analysis, the KT_{50} (50% knockdown time) was determined. Using Duncan's Multiple Range Test (DMRT), mortality statistics were examined.

The following is an Effective Knockdown Index (EKI) to effectively demonstrate how much greater or less effective a formulation was compared to cypermethrin in terms of knockdown efficacy,

$$\text{Effective Knockdown Index (EKI)} = [KF/KC],$$

whereas KF represented the KT_{50} of each formulation, and KC represented the KT_{50} offered by 1% cypermethrin.

Hence, EKI < 1 would therefore imply that 1% cypermethrin was not more hazardous than the individual EO, EO ingredient, or combination formulation (KT_{50} value is a time value, so the shorter the better); EKI > 1 would suggest the opposite; and EKI = 1 would indicate similar toxicity.

In the same vein, to efficiently show how much more or less effective each formulation was compared to 1% cypermethrin in terms of mortality, we established an Effective Mortality Rate Index (EMI). EMI was estimated using the following formula,

$$\text{Effective Mortality Rate Index (EMI)} = [MF/MC],$$

where MF represented each formulation's mortality rate and MC represented cypermethrin's mortality rate.

However, unlike the meaning of EKI, EMI < 1 signified that the formulation was less effective at killing houseflies than 1% cypermethrin; EMI > 1 would suggest the opposite, and EMI = 1 would suggest similar killing efficacy.

Results

Susceptibility test

It displayed the knockdown rate, KT_{50}, and Effective Knockdown Index (EKI) of each individual EO, each EO constituent, and their combined formulations against the housefly (*M. domestica*) (Table 2). The most effective individual EO and EO constituents were 5% *C. citratus* EO, followed by 1% geranial, 1% eucalyptol, and 5% *E. globulus* EO. Furthermore, 2% *E. globulus* EO + 1% geranial and 2% *C. citratus* EO + 1% eucalyptol combined formulations were even more effective than 5% of individual *C. citratus* EO the first formulation provided a KT_{50} of 4.27 min, and the second formulation provided a KT_{50} of 5.85 min, while 5% *C. citratus* EO provided a longer KT_{50} of 6.69 min. Moreover, the knockdown rates provided by 2% *E. globulus* EO + 1% geranial and 2% *C. citratus* EO + 1% eucalyptol 2% were better than that provided by cypermethrin. Regarding EKI, all formulations were better than 1% cypermethrin, except 1% eucalyptol.

The mortality rate, WHO susceptibility status, and Effective mortality Index (EMI) for all formulations used to treat houseflies (*M. domestica*) was listed in Table 3. The most effective individual EO and EO constituents in terms of mortality rate were 5% *C. citratus* EO, followed by 1% geranial, 1% eucalyptol, and 5% *E. globulus* EO, having a mortality rate that ranges from 0 to 100%. The combined 2% *C. citratus* EO + 1% eucalyptol and 2% *E. globulus* EO + 1% geranial formulations were as effective as 5% *C. citratus* EO, assuming 100% mortality, and more effective than 1% cypermethrin (56.7%). Regarding EMI, all formulations were better than 1% cypermethrin, except 5% *E. globulus* EO and 1% eucalyptol.

Table 2. Housefly (*M. domestica*) knockdown rate (KT_{50}) and effective knockdown index (EKI) after 60 min following exposure to eucalyptol, geraniol, *C. citratus*, and *E. globulus* essential oils, as well as their combination formulations

Treatment	Knockdown rate ±SD	KT_{50} (min)	EKI
5% *C. citratus* EO	100 ±0 [a]	6.69	0.16
5% *E. globulus* EO	0 [c]	na	-
1% eucalyptol	8.0 ±19.30 [c]	113.76	2.79
1% geranial	94.00 ±9.85 [a]	15.62	0.38
2% *C. citratus* EO + 1% eucalyptol	100 ±0 [a]	5.85	0.14
2% E. *globulus* EO + 1% geranial	100 ±0 [a]	4.27	0.10
1% cypermethrin	56.70 ±32.40 [b]	40.78	-
70% ethyl alcohol	0 [c]	na	-

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

Table 3. Housefly (*M. domestica*) Mortality rate, WHO susceptibility status, and Effective Mortality Index (EMI) after 24 h following exposure to eucalyptol, geranial, *C. citratus*, and *E. globulus* essential oils, as well as their combination formulations

Treatment	Mortality rate ±SD	Susceptibility	EMI
5% *C. citratus* EO	100 ±0 [a]	S	1.76
5% *E. globulus* EO	0 [c]	R	-
1% eucalyptol	10.70 ±18.70 [c]	R	0.19
1% geranial	94.66 ±9.90 [a]	S	1.67
2% *C. citratus* EO + 1% eucalyptol	100 ±0[a]	S	1.76
2% *E. globulus* EO + 1% geranial	100 ±0 [a]	S	1.76
1% cypermethrin	56.70 ±32.40 [b]	R	-

WHO status: S = Susceptible is defined as having a mortality rate of 98–100%, PR = Possible Resistant as having a mortality rate of 80–97%, and R = Resistant as having a mortality rate of 80%;
EMI= Effective Mortality Index.

Discussion

There are three key topics covered. The first major argument is that the combined 2% *E. globulus* EO + 1% geranial formulation provided a better knockdown rate against *M. domestica* than 1% cypermethrin, implying that this formulation can replace cypermethrin. Before we began the study, it was anticipated that this formulation would have high efficacy due to the efficacy of each individual formulation. *E. globulus* EO with eucalyptol main constituent and geranial, has been reported to be high by several earlier investigations: Kumar *et al*. (2014), Soonwera and Sittichok (2020).

The second main point is that 2% *E. globulus* EO + 1% geranial formulation provided a better knockdown rate against *M. domestica* than 2% *C. citratus* EO + 1% eucalyptol, implying that geranial was more synergistic with *E. globulus* EO than eucalyptol with *C. citratus* EO. Both studies reported Scalerandi *et al.* (2018) and Soonwera and Sittichok (2020) the existence of this kind of synergy between EOs and their EO constituents, indicating that, to a certain extent, our techniques and results were reliable.

The third and final main point is the safety of *E. globulus* and geranial. Poisoning by *E. globulus* has been reported by Ittyachen *et al.* (2019). Indian men who accidentally consumed eucalyptus oil were taken to emergency room with seizures, which were caused by fake eucalyptus oil that may contain camphor. However, eucalyptus oil taken from eucalyptus tree does not contain camphor, and consumption in moderate amounts will not cause toxicity. Lillian C. Becker reported the lethal oral dose for human adults as 0.05 mL to 0.5 mL/kg of pure eucalyptus oil (Becker, 2017). Regarding geranial, it can be toxic to human blood cells at high concentration, with an LC_{50}, directly applied in vitro to blood cells, in a range between 146.12 to 433.15 µg/mL (Hacke *et al.*,2022), implying that 2% E. globulus EO + 1% geranial at this low concentration of geranial is safe. Nevertheless, a complete development of this formulation into commercial product will need to include a full-scale battery of safety tests.

As a result of our research, we can say that the combination of 2% E. globulus EO and 1% geranial has tremendous promise as a safer yet currently more effective pesticide than the widely used synthetic insecticide cypermethrin.

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