International Journal of Agricultural Technology 2025 Vol. 21(2):555-562
DOI: https://doi.org/10.63369/ijat.2025.21.2.555-562
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Plant essential oils, trans-anethole and eugenol, for housefly knockdown and mortality

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Sittichok, S., Passara, H., Sinthusiri, J., Soonwera, M., Thongsaiklaing, T., Morris, J., Moungthipmalai, T., Puwanard, C. and Jintanasirinurak, S. (2025). Plant essential oils, trans-anethole and eugenol, for housefly knockdown and mortality. International Journal of Agricultural Technology 21(2):555-562.

Abstract The knockdown and mortality of some compounds from natural oils (trans-anethole, eugenol and a combination of trans-anethole and eugenol) against adult houseflies (*Musca domestica*) compared with cypermethrin was investigated. The knockdown and mortalities were highest at 100% of 1% trans-anethole with KT_{50}= 31 min and LT_{50} = 31 min. The mortality index of trans-anethole was 82.1 times that of cypermethrin. In contrast, 1% eugenol had knockdown and mortality was 36% and 0.5% trans-anethole + 0.5% eugenol had knockdown and mortality of 68%, with mortality indexes of 1.6 and 3.2 times versus cypermethrin. The trans-anethole high toxicity and low residue made it a superior control for houseflies.

Keywords: Plant essential oils, Trans-anethole, Knockdown, Mortality, *Musca domestica*

Introduction

Musca domestica, the common housefly, is a common insect pest of the family Muscidae and the order Diptera. The adult fly is drab gray with a yellowish abdomen and a longtitudinal line along the thorax; its body 5-7 mm body long and its compound eye has 4,000 facets. The adult female produces 100–5000 eggs in its life span. It is well-adapted to human and animal habitats

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such as houses, hospitals, restaurants and farms (Khamesipour *et al*., 2018). It carries pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Shigella* sp., *Salmonella* sp. and *Vibrio cholera*, which are normally found in dung. It can cause major diseases in humans and domestic animals, including bacillary dysentery, typhoid, paratyphoid, salmonellosis, shigellosis, anthrax and myiasis (Sinthusiri and Soonwera, 2014).

At present, the synthetic chemicals have effectively controlled the fly population. Pyrethroids, organophosphates and carbamate chemical insecticides can be used in various forms, such as coils, aerosol sprays and fumigants. However, using chemical insecticides for a long period results in mutation and an increase in the pest insecticide resistance, leading to difficulty in pest control. Chemical insecticides and their residues that remain in the environment also pose risks to non-target organisms – some of which are essential components of the natural environment (Freeman *et al*., 2019; Soonwera and Sittichok, 2020; Wang *et al*., 2019).

For all these reasons, plant essential oils (plant EOs) are the new agents for controlling the housefly population. They can be used to control insect pests in many countries (Patel *et al*., 2022; Begna, 2015). Some people used plant EOs as an alternative to reduce the disadvantage of using chemical insecticides. They were used as an alternative to chemical insecticides such as repellent, antifeedant, mortality, and growth inhibition; additionally, they could reduce insect pest resistance while rapidly degrading (Mochiah *et al*., 2011). The main chemical compounds in plant essential oils have insecticidal activity for pest control. Trans-anethole is the main chemical compound in the dried fruit of star anise EO (Gholivand *et al*., 2009). It was an effective insecticide with an effect on acetylcholinesterase activity in pests (Cruz *et al*., 2013). Eugenol in clove oil from the dry flower bud had a neurotoxic mechanism affecting acetylcholinesterase and octopamine synapses, which control insect pests (Regnault *et al*., 2012).

This study evaluated the effectiveness against adult flies (*M. domestica*) of chemicals in several plant-sourced essential oils.

Materials and methods

Materials

Table 1. Materials used in this study are listed

Material	Source	Notes
Adult flies	Nongchok market, Thailand	13.8557° N, 100.8625° E
Glucose syrup	Mitr Phol, Thailand	Sucrose 50%, glucose 25%, fructose 35%
Dog food	Mars Petcare, Thailand	Pedigree
Milk powder	Nestle (Thai) Co., Ltd., Bangkok, Thailand	Carnation
Steamed Mackerel fish	Central Food Retail.Co. Ltd., Bangkok, Thailand	Tops
Coconut husks	Home Pro, Bangkok, Thailand	Purchased in sterilized packets
Trans-anethole	Sigma-Aldrich, USA	CAS 4180-23-8, 99%, complying with HACCP
Eugenol	Sigma-Aldrich, USA	CAS 97-53-0, 99%, complying with HACCP
Nonyl phenol ethoxylate	Chemipan Co., Ltd., Bangkok, Thailand	NP9, Surfactant, HLB = 12.9
Cypermethrin	Pentacheme Co., Ltd., Bangkok, Thailand	Chemical insecticide

Main chemical compound EO preparation

The main chemical compounds (EOs) of star anise (trans-anethole) and cloves (eugenol) were purchased as 98% pure compounds. They were diluted with water and nonyl phenol ethoxylate (NP9 or Tergitol) as surfactant. Cypermethrin was used as a positive (active) control, and NP9 was used for negative (inactive) control.

Knockdown and mortality assay

The knockdown (or inactivity) on adult flies was tested with filter paper treated with the oil for 1 hour in a tube and then transferred to another tube. Knockdowns were counted at for 5, 10, 30 and 60 minutes: 5 replicates were used. The knockdown was not moved for 60 minutes. Mortalities were recorded at 1440 minutes and calculated from:

$$\text{Knockdown rate (\%K)} = KD/TN \times 100$$

where KD is the number of knocked down (on inactive) flies and TN is the number of treated flies.

Then, at 24 hours, mortality was compared with positive and negative controls. No movement for 1440 minutes was considered dead. Mortality (%M) was calculated from:

$$\text{Mortality rate (\%M)} = MT/TN \times 100$$

where MT is the total number of dead flies; and TN is the total number of treated flies.

The mortality index was calculated from:

$$\text{Mortality index} = LT_{50}C / LT_{50}T$$

where LT_{50} C is the LT_{50} of the cypermethrin and LT_{50} T is the LT_{50} of the tested nanoemulsion.

Statistical analysis

These experiments used a completely randomized design (CRD). The 50% and 90% knockdown times (KT_{50} and KT_{90}) and the 50% and 90% lethal times (LT_{50} and LT_{90}) were calculated using standard probit analysis. Mortality data was analyzed with Duncan's Multiple Range Test (DMRT).

Results

Trans-anethole and eugenol alone, 'Formula I' (0.5% trans-anethole and 0.5% eugenol) and 1% cypermethrin and NP9 as controls were tested. The knockdown of trans-anethole against the adult flies showed 100% knockdown at 1% trans-anethole. However, 1% eugenol alone showed only 35%, whereas the combination of 0.5% trans-anethole with 0.5% eugenol showed 68%, and the chemical insecticide (1% cypermethrin) showed only 14% knockdown at 1 hour (Table 2). The relevant KT_{50} and KT_{90} values are shown in Table 3.

Table 2. Knockdown of houseflies versus chemical

Emulsion	Average knockdown fraction (mean (%) ± SD)					
	Time (minutes)					
	1	5	10	15	30	60
Trans-anethole	$0.0{\pm}0.0^{na}$	$0.0{\pm}0.0^{na}$	$22.0{\pm}4.5^{a}$	$22.0{\pm}4.5^{a}$	$32.0{\pm}4.5^{a}$	$100.0{\pm}0.0^{a}$
Eugenol	$0.0{\pm}0.0^{na}$	$0.0{\pm}0.0^{na}$	$0.0{\pm}0.0^{b}$	$0.0{\pm}0.0^{c}$	$10.0{\pm}0.0^{b}$	$36.0{\pm}8.9^{c}$
Formula I	$0.0{\pm}0.0^{na}$	$0.0{\pm}0.0^{na}$	$0.0{\pm}0.0^{b}$	$10.0{\pm}0.0^{b}$	$10.0{\pm}0.0^{b}$	$68.0{\pm}4.5^{b}$
Cypermethrin	$0.0{\pm}0.0^{na}$	$0.0{\pm}0.0^{na}$	$0.0{\pm}0.0^{b}$	$0.0{\pm}0.0^{c}$	$0.0{\pm}0.0^{c}$	$14.0{\pm}5.5^{d}$
NP9	$0.0{\pm}0.0^{na}$	$0.0{\pm}0.0^{na}$	$0.0{\pm}0.0^{b}$	$0.0{\pm}0.0^{c}$	$0.0{\pm}0.0^{c}$	$0.0{\pm}0.0^{e}$

Remark: Trans-anethole and eugenol emulsion formula: formula I

Note: Knockdown rates within a column followed by a different letter differ significantly by DMRT at $p<0.05$.

Table 3. KT_{50} and KT_{90} values for the major constituents

Emulsion	KT_{50} (min) (Lower-Upper)	KT_{90} (min) (Lower-Upper)	Slope±SE	χ^2
Trans-anethole	31 (27-35)	50 (44-58)	0.067±0.007	27.1
Eugenol	67 (59-78)	95 (82-118)	0.045±0.007	4.2
Formula I	51 (46-57)	76 (68-88)	0.052±0.006	10.5
Cypermethrin	72(-)	86(-)	0.091±0.145	1.0
NP9(Control)	*na*	*Na*	0.0	0.0

Remark: KT_{50} = 50% Knockdown time; KT_{90} = 90% Knockdown time; *na*: not computable by probit analysis

A 1% concentration of trans-anethole had the highest (100%) mortality, whereas 1% eugenol killed only 36% and formula I (0.5% trans-anethole + 0.5% eugenol) killed 68% (see Figure 1). However, for 1% cypermethrin, it was only 18%. Corresponding LT_{50} and LC_{90} values at 1440 minutes are in Table 4. Clearly, trans-anethole was mostly effective against adult houseflies.

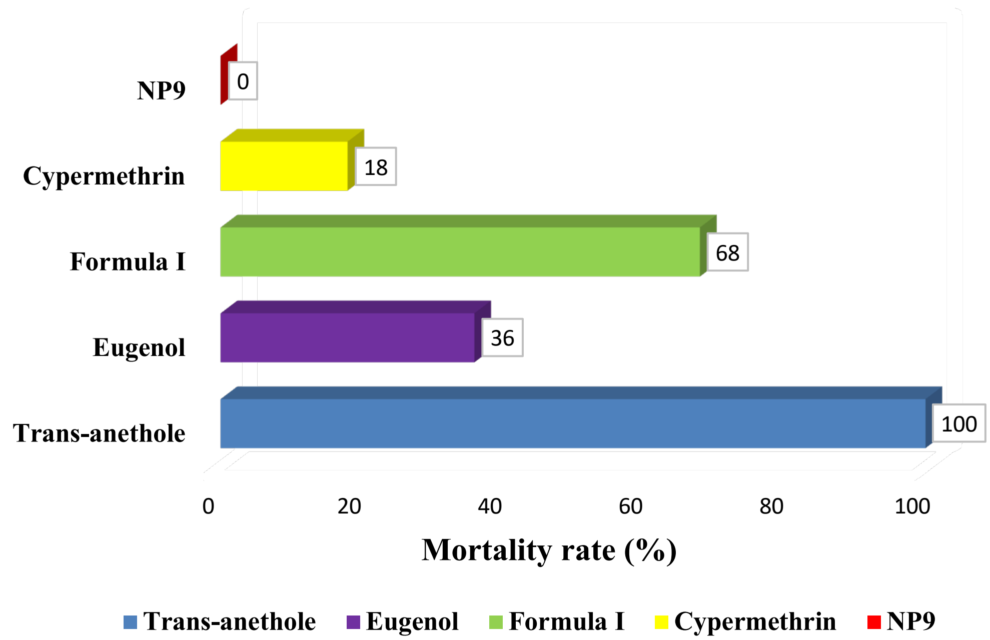

Figure 1. Housefly mortality vs various chemicals at 1440 minutes

Table 4. LT_{50}, and LT_{90} for houseflies after exposure

Emulsion	LT_{50}(min) (Lower-Upper)	LT_{90}(min) (Lower-Upper)	Slope±SD	χ^2	Mortality index
Trans-anethole	31 (27-35)	50 (44.1-58.3)	0.067±0.007	27.1	82.1
Eugenol	1611 (1153-2741)	2949 (2102-5202)	0.003±0.001	86.2	1.6
Formula I	789 (540-1282)	1626 (1174-2685)	0.002±0.001	149.9	3.2
Cypermethrin	2520 (1873-4077)	4069 (2976-6785)	0.004±0.001	46.9	-
NP9(Control)	*na*	*Na*	0.0	0.0	-

Remark: LT_{50} = 50% Lethal time; LT_{90} = 90% Lethal time; *na*: not computable by probit analysis

Discussion

In the present study of chemicals from plant essential oils, only one compound, trans-anethole (derived from star anise), showed strong insecticidal activities against adult houseflies measured by knockdown, $KT_{50} = 31$ min, and mortality, $LT_{50} = 31$ min. The mortality index showed it was more than 82 times more effective than cypermethrin. Similarly, trans-anethole strongly repelled flour beetles (Alkan and Ertürk, 2020) and was highly toxic to the 4^{th} instar larvae of fall webworm with $LC_{50} = 1.41$ µl/mL (Pour *et al*., 2022). Additionally, previous studies showed that 1% lemongrass + 1% trans-anethole and 1% geranial + 1% trans-anethole were highly effective against houseflies, and led to mortality of more than 90% (Soonwera *et al*., 2024). In contrast, star anise EO + geranial, nutmeg EO + geranial and α-pinene + geranial had only slightly more than 50% mortality (Aungtikun *et al*., 2021). trans-anethole + estragole and trans-anethole + estragole + linalool had strong synergistic activity against the rusty grain beetle (Zibace and Khorram, 2015).

Previous work showed that trans-anethole in star anise was a secondary metabolite and was highly toxic to insect pests (Hikal *et al*., 2017) and was found not to have residual toxicity (Alkan and Ertürk, 2018). It inhibited AChE activity (Wang *et al*., 2021) and inibited digestion: further, it induced detoxifying activity and antioxidant enzymes in fall webworms (Pour *et al*., 2022). It also caused increased physiological disorders in the larvae of Mediterranean flour moths by inhibiting ACh and causing an imbalance of antioxidants (Shahriar *et al*., 2018). Thus, trans-anethole had a similar action as a chemical insecticide, but had fewer environmental side effects.

Chemical insecticides are generally used against insect pests. The efficacy of chemical insecticides was generally 90% in first pest generation (Dong *et al*., 2007). Here, we showed that the main chemicals in plant oils had higher knockdown and mortality than a chemical insecticide, like cypermethrin. However, now cypermethrin has a low effect, since it takes a long time and the pests could develop resistance to it. Thus, botanical insecticides for alternative pest control have significant benefits over chemical ones (Gitahi *et al*., 2021) due to resistance and are non-toxic in the environment and, specifically, to beneficial organisms (Batiha *et al*., 2020).

In conclusion, trans-anethole increased the efficacy of housefly control and had benefits as a botanical insecticide to reduce or eliminate the use of chemical insecticides, because it had no human or animal side effects or ecosystems, in general, because of fast natural degradation (Mahanta *et al*., 2021).

Acknowledgments

This work is supported by King Mongkut's Institute of Technology Ladkrabang **(**KMITL**)**, Bangkok 10520, Thailand. (grant number KREF046703).

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(Received: 12 September 2024, Revised: 2 March 2025, Accepted: 10 March 2025)