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Adulticidal activity of star anise, turmeric, cloves and combinations against houseflies

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Abstract The small droplet size could increase the absorbed ingredients and high stability. The star anise nanoemulsion was determined optimum efficacy, with a 1% concentration resulting in the highest knockdown and mortality rate of 100%. Its KT_{50} and mortality effective values were 28 min and 36 times, respectively. Furthermore, the adult stage of houseflies is found to be susceptible to star anise nanoemulsion. In contrast, cypermethrin was less toxic to it, with a high KT_{50} and resistance to adult houseflies. Consequently, the star anise nanoemulsion could be developed into an efficient and safe environment for controlling housefly populations.

Keywords: Housefly, Insecticidal activity, Knockdown, Mortality, Star anise

Introduction

The housefly (*Musca domestica* L.) is a ubiquitous but dangerous insect. Its remarkable features are the bristles covering the body, sponging mouthparts and compound eyes (Iqbal *et al*., 2014). It is a pest on farms and in human habitation. Its control is important, because it carries pathogens bacteria, protozoa and viruses to both livestock and humans, as it feeds on and breeds in decomposing matter and human excretion (Hasan and Leong, 2018): this makes it both annoying and a health risk (Khamesipour *et al*., 2018). It has been a

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problem continuously because of its short lifespan and insecticide resistance (WHO, 2018).

Chemical insecticides are commonly used for controlling houseflies, because it is an easy way to remove them. However, these flies are developing resistance to chemical insecticides and chemical residues in the environment (Khamesipour *et al*., 2018; Soonwera and Sittichok, 2020; Wang *et al*., 2019). Now, botanical insecticides represent an alternative that reduces the negative effects of using chemical insecticides: they can be used to control flies and are environmentally friendly (Sinthusiri and Soonwera, 2014). These insecticides, sourced from essential oils, can be used as an alternative to chemical insecticides, because they act as repellents, insecticidal and ovicidal agents, growth inhibitors and inhibit feeding, and others. Further, they can reduce resistance to commonly used insecticides, while rapidly degrading (Chaudhari *et al*., 2021; Isman, 2006; Mochiah *et al*., 2011).

Botanical nanoemulsions have become an alternative to reduce insect pest problems. Nanoemulsions are secondary metabolites that plants produce to defend themselves in unsuitable environments and also destroy atttacking insect pests. Nanoemulsions are generally stable, easily formed, have low volatility and remain soluble during storage. Thus, nanoemulsions can be developed into highly effective insecticides (Noichinda and Suppavorasatit, 2019).

This study aimed to develop eco-friendly insecticides in the form of plant essential oil nanoemulsions for controlling houseflies that can be practically applied successfully in the domestic and rural environments.

Materials and methods

M. domestica Breeding

The adult houseflies were collected manually from a market in Lat Krabang and reared in a 300×300x300 mm^3 laboratory container at room temperature (25±2 °C) at the Laboratory of Entomology and Environment, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. They were fed with 10% syrup soaked in cotton and dog food mixed with milk powder. Coconut husks were sterilized by the manufacturer and used as supplied. At 300 g mass, mackerel fish with sterile coconut husks were placed in a plastic box for laying eggs and food for the fly larvae. Newly born adults were used for this study. These materials used in this study are listed in Table 1.

Table 1. Materials used in this study are listed

Material	Source	Notes
Adult flies	Yeam Charoen Rat market	13.7234° N, 100.7841° E
Glucose syrup	Mitr Phol, Thailand	Sucrose 50%, glucose 25%, fructose 35%
Dog food	Mars Petcare. Thailand	Pedigree
Milk powder	Nestle (Thai) company limited	Carnation
Steamed Mackerel fish	Central Food Retail.Co. Ltd Home Pro, Thailand	Tops Purchased in sterilized packets
Star anise Turmeric Cloves	Thai-China Flavors and Fragrances Industry Co. Ltd., Thailand	Essential oil
NP9 Tween 20 Tween 80	Thai-China Flavors and Fragrances Industry Co. Ltd., Thailand	Surfactant

Plant nanoemulsion essential oils preparation

The plant nanoemulsions were prepared using three surfactants and co-surfactants (NP9, Tween 20, and Tween 80) as shown in Table 2. These EOs were highly soluble and did not precipitate when formed. After that, the emulsions were sampled and measured for particle size and zeta potential with a particle analyzer (Manufacturer, type). Particle sizes of the nanoemulsions ranged from 13.3 to 61.1 nm: all were < 100 nm; zeta potentials ranged from -8.06 to -17.66 mV (Table 2).

Table 2. Particle sizes of essential oils with different ratios of surfactants

Emulsion	Surfactant	Co-surfactant	Ratio	Particle size(nm)	Zeta potential(mV)
Star anise	NP9	Tween 20	1:3:3.5	13.3	-10.07
Turmeric	NP9	Tween 20	1:2:1	61.6	- 8.06
Cloves	NP9	Tween 80	1:2.5:3	14.2	-17.56
Mixed formulations					
Formula I	Star anise	Turmeric		35.6	-6.30
Formula II	Star anise	Cloves		43.2	-1.67
Formula III	Turmeric	Cloves		14.2	2.03

Remark: Ratio of each component is shown in the column to the right.

Bioassay test-laboratory conditions

Adult flies were tested with filter paper treated nanoemulsions for 1 hour in a stoppered tube then transferred to another tube. Knockdown rates were recorded at 1, 5, 10, 15, 30 and 60 minutes. Mortality was observed after 24 hours and the mortality rates were calculated and compared with the control group. Each test used five replicates. The knockdown rate and mortality rate were calculated from:

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

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

where KD = total number of knockdown adult flies, MT = total number of dead adult flies, TN = total number of treated adult 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

The data were analyzed using standard probit analysis to obtain KT_{50}, KT_{90}, LT_{50}, and LT_{90}. Knockdown and mortality data were analyzed using one-way ANOVA and data means with compared by Duncan's Multiple Range Test (DMRT). Levels of susceptibility followed WHO criteria (WHO, 2018):

98-100% mortality = susceptible,
80-97% mortality = possible resistance, and
< 80% mortality = resistance.

Results

Adulticidal bioassay

Toxicity of the six selected plant nanoemulsion are listed in Table 3 and Figure 1 against adult flies showed that the 1% concentration of star anise nanoemulsion was the most effective with 100% fly knockdown at 1 hour: toxicities of all tested emulsions are shown graphically in Figure 1. The single components were more effective than cypermethrin (14%), but the combinations were significantly more effective (>54%) than cypermethrin (Figure 1). However, for the adult flies, all KT_x (x = 50 or 90) values were lower than those for cypermethrin (Table 3). The NP9 (negative control) naturally did not cause any deaths.

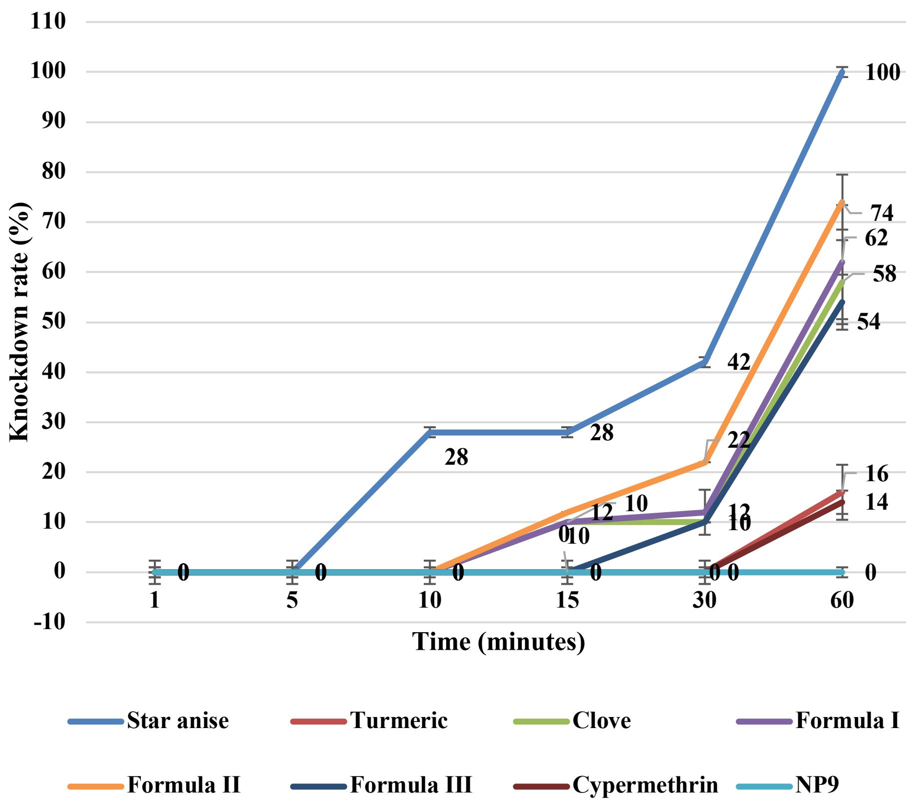

Figure 1. Average fly knockdown rates for the tested nanoemulsions
Note: See Table 2 for formula definitions

World Health Organization (WHO) susceptibility test

For mortality, the star anise nanoemulsion at 1% concentration had the highest mortality at 100%. Similarly, star anise with turmeric and with clove nanoemulsions were extremely effective at 100% killing at 24 hours, compared with the positive control (cypermethrin) at 18%. From LT_{50} values, flies were susceptible to the star anise nanoemulsion, $LT_{50} = 28$ min, thus cypermethrin was 36 times less effective – taking much longer times, thus cypermethrin showed fly resistance with $LT_{50} > 980$ min (Table 4).

Table 3. Knockdown times: KT_{50} and KT_{90} for the various nanoemulsions

Nanoemulsion	KT_{50} (min)	KT_{90} (min)
Star anise	28	46
Turmeric	71	85
Clove	55	83
Formula I	53	80
Formula II	46	71
Formula III	54	78
Cypermethrin	72	86
NP9	NA	NA

Remark: KT_x = time for x% knockdown;NA: no flies died using the control (NP9)

Table 4. Mortalities, LT_{50} and LT_{90}, and susceptibility of flies to nanoemulsions

Nanoemulsion	Mortality rate±SD	LT_{50}(min)	LT_{90}(min)	Susceptibility	Mortality index
Star anise	100.0 ± 0.0^{a}	28	46	S	36
Turmeric	20.0 ± 0.0^{d}	2413	3936	R	0
Clove	82.0 ± 8.4^{b}	871	1742	PR	1
Formula I	100.0 ± 0.0^{a}	492	993	S	2.0
Formula II	100.0 ± 0.0^{a}	46	71	S	21
Formula III	72.0 ± 5.5^{c}	54	71	R	18
Cypermethrin	18.0 ± 4.5^{d}	982	1896	R	-
NP9	0.0 ± 0.0^{e}	NA	NA	-	-

Remark: See Table 1 for formula definitions;
LT_{50} = 50% Lethal time; LT_{90} = 90% Lethal time;
NA: The control led to 0 mortality

Discussion

This study showed that the plant nanoemulsions were effective insecticides against adult flies. In particular, the 1% concentration of star anise had a higher knockdown rate, mortality rate, higher toxicity and susceptibility to flies than a typical synthetic insecticide, cypermethrin. In general, the tested combinations had shorter knockdown time (KT_{50} and KT_{90}) and lethal time (LT_{50} and LT_{90}) than cypermethrin. Additionally, these results are consistent with previous studies that 0.5%-star anise and 0.5% geranial had the highest synergies against houseflies - at 100% knockdown and mortality rates and LT_{50} = 6.0 min (Aungtikun *et al*., 2021). Also, 1% lemongrass EO with 1% trans-anethole and 1%-star anise with 1% geranial combinations showed 94 - 96% actions against fly eggs (Passara *et al*., 2024). Star anise also showed high insecticidal activity against the larvae and adult stages of the rust grain beetle (Wang *et al.,* 2021): it also repelled housefly (Sinthusiri and Soonwera, 2014). It was also fatal to cherry vinegar fly (Kim *et al*., 2016) and exhibited a high activity against mosquito eggs at 100% and an LC_{50} of 1.0 to 1.4% (Puwanard and Soonwera, 2022).

These nanoemulsions were prepared by aqueous titration with surfactants as emulsifiers for the distribution as droplets (Ariyaprakai, 2017). This method was cheap when compared to the other methods and it was an easy to manually stir the mixtures of plant EOs, surfactants and water (McClement and Rao, 2011). The droplet size was 13.3 nm < 100 nm, consistent with Kumar and Kumari (2019). The zeta potential of the star anise nanoemulsion was -10.07 mV - an indicator of stability. A negative zeta value could induce repulsive forces stronger than the attractive force in each droplet (Mohammadi *et al*., 2022). The plant nanoemulsions used botanical insecticides. These nanoemulsions were not easily degraded by air, light and temperature and reduced the disadvantages of emulsions (Isman, 2020). The formulation had good homogeneity and stability in droplet size (Jintapattanakit, 2018). Thus, small plant nanoemulsions were more effective as botanical insecticides than larger ones because they were more readily absorbed.

Cypermethrin, a synthetic pyrethroid insecticide (Crawford *et al*., 1981) was reported to induce the development of neurotoxicity and blocked nerve impulses by stopping sodium ion transport channels in the nerve membranes (Singh *et al*., 2012). In this study, it was less effective against houseflies. The measured mortalities of these star anise, and the combinations (formula I – III) nanoemulsions were more effective. Many countries have used chemical insecticides that affected the water and soil. Previously, they were highly effective in pest control, but, now, with continued use, insects have developed resistance to them (Duhan *et al*., 2017). They have become out of control. Additionally, the residue of chemical insecticides can negatively affect human health and the environment. Thus, botanical insecticides are considered alternative agents for pest control, since they degrade quickly in the environment with low toxicity to mammals and have a lower risk of resistance to insect pests.

In conclusion, star anise was toxic to adult flies; it was environment-friendly and thus the nanoemulsion was an important alternative for controlling houseflies and reducing their health risks.

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