
Impact of botanical essential oils on pupation and survival of *Musca domestica* L.

Moungthipmalai, T.¹, Sriboonjit, N.², Khuendee, P.³, Phunglakchai, J.⁴, Thongsaiklaing, M.⁵, Soonwera, M.^{1*} and Passara, H.¹

¹Office of Administrative Interdisciplinary Program on Agricultural Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand; ²Sarasas Witaed Suksa School, Samut Prakan, 10290, Thailand; ³Demonstration School of Ramkhamhaeng University, Bangkok, 10240, Thailand; ⁴Debsirin School, Bangkok, 10100, Thailand; ⁵Faculty of Agriculture, Princess of Naradhiwas University, Narathiwat, 10540, Thailand.

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Abstract Among the tested botanical extracts, *Illicium verum* (star anise) essential oil at 10% demonstrated the highest efficacy in disrupting normal pupal development. Only 65.00% of individuals successfully emerged as fully developed adults after a 10-day exposure period. A notable proportion exhibited transitional deformities, particularly malformed pupal-adult intermediates, indicating interference with metamorphic progression. In comparison, cypermethrin, a commonly applied synthetic insecticide at the same concentration, resulted in a lower mortality rate of 39.67% under identical experimental conditions. The superior performance of star anise oil highlighted its potential as a plant-derived alternative for insect control. These findings suggested that the incorporation of such natural products into integrated pest management strategies may reduce dependence on conventional synthetic chemicals.

Keywords: Botanical essential oil, Insecticidal activity, Mortality, *Musca domestica*, Pupation

Introduction

The housefly (*Musca domestica* L.) is a ubiquitous insect species, recognized as an improver of disease transmission. It is known for its hair-covered body, sponge-like feeding organs, and many-faceted eyes (Iqbal *et al.*, 2013). This household nuisance persists in urban and agricultural areas, creating a great nuisance. It also carries microorganisms, such as bacteria, protozoans, and viruses. It breeds in waste and decaying substances that can spread to animals and people (Abu Hasan and Leong, 2018), so controlling its population is essential. Consequently, it isn't just an annoyance and also represents a significant public health challenge (Khamesipour *et al.*, 2018). It's due to the cyclical nature of reproduction and resistance to chemical control mechanisms

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

that continue posing a challenge for its management (WHO, 2018). They often alight on people's faces, arms, and food, and their breeding sites can produce strong foul odors. This makes the environment not pleasant for residents or tourists (Moungthipmalai and Soonwera, 2022).

Many artificial pest control agents have been found effective against housefly infestations (Moungthipmalai and Soonwera, 2022). Cypermethrin is an artificial technical insecticide that was developed more than forty years ago to safeguard humans and animals against problematic insects (Sinha and Shrivastava, 2024). However, the use of these chemical agents in modern times is fraught with problems, such as pest resistance development, ecosystem pollution, hazard to nontarget organisms and toxicity to people (Zhang *et al.*, 2022). In this regard, essential oils derived from plants have been explored and advertised as safer and less environmentally toxic alternatives to traditional synthetic chemical insecticides (Chen *et al.*, 2018).

Plant-based nanoshiftres such as those of nanoemulsions have been established to be a viable option in controlling pest infection (Passara *et al.*, 2024). These nanoscale formulations are stored secondary plant compounds that help defend against environmental stresses and invading insects. They are characterized by their high physical stability, ease of preparation, low evaporation rate and the mixture remain emulsified over time (Montes de Oca Avalos *et al.*, 2017). Because of these benefits, they are regarded as excellent candidates for the development of powerful bioinsecticides (Rehman *et al.*, 2020).

This study aimed to develop environment friendly insecticides from nanosized emulsified plant essential oils against housefly pupae that can have utility at household and rural levels.

Materials and methods

Collection and maintenance of *Musca domestica* pupae

Fully-grew *M. domestica* were captured by net from a wholesale market in Lat Krabang and placed into a 300 × 300 × 300 mm³ cage environment at the Laboratory of Insect Studies and Environmental Research, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok (temperature: 25.0 ±2.0 °C; relative humidity: 75.0 ±3.0%). Insects were given 10% sucrose solution in 50 mL bottle drops by cotton balls and dry canine feed mixed with milk powder. Commercial sterile coconut husk that was processed and packaged by the manufacturer was used without additional sterilization. To support the growth of larvae, 300 g mackerel were combined

with sterile coconut husk and placed in a container as both an oviposition substrate and food for larvae. Subsequently, newly formed pupae were isolated and underwent further experimental protocol procedures.

Development of plant-based essential oil nanoformulations

The other half of the formulations contained oil derived from plant and used as emulsifiers combination NP9 + Tween 20. The oil mixtures were noted to have good dispersion properties and showed no signs of sedimentation or layer separation. Following preparation, droplet diameter and electrokinetic potential was assessed by nanoparticle measurement device (NanoPlus Zeta, Otsuka Electronic Co., Ltd., Osaka, Japan) Droplet sizes were between 23.5 and 34.9 nm which confirmed the definition of all samples to be nanoscale systems (<100 nm). The zeta potential values measured from -18.78 to 12.02 mV indicated that the emulsions had moderate electrostatic stability.

Pupation bioassay

This experiment was conducted using a Completely Randomized Design (CRD) with 3 replications. Ten one-day-old house fly pupae on glass dish (5-cm diameter) was considered as one experimental unit. The procedure consisted of laying them on a glass dish, and then 10 microliters of an herbal essential oil of each type and concentration (1%, 5%, 10%) was applied on each pupa. The dishes were then placed inside 7.5 x 10 x 5 centimeter plastic boxes for daily observation of pupae development over a period of 10 days. To statistically validate the results, we repeated the experiment two more times. NP9 was established as a negative control and α -cypermethrin was used as a positive control, with all operations identical to those used for the herbal essential oil treatments.

Statistical data analysis

Data were subjected to analysis of variance and the differences among means at each concentration of each tested substance were accomplished by Duncan's Multiple Range Test (DMRT). Acute toxicity was evaluated using the lethal concentration for 50% and 90% mortality (Lethal concentration 50 and 90: LC₅₀ and LC₉₀), estimated by Probit analysis.

Results

Pupal toxicity and developmental disruptions against M. domestica pupae induced by medicinal plant-derived volatiles

As such, the results revealed the toxic effects of three medicinal plants nanoemulsion (administered to *M. domestica* at 1%, 5% and 10%) on mortality pupa information and morphophysiological in adults (Table 1). The three-novel plant-derived nanoemulsions all induced pupa survival and development, while the effects were the most potent at 10% then depending on potency by 5%, before showing lowest at a concentration of 1%. Mature normal adult flies were emerged successfully with percentage (65.00%) lowest at 10% concentration of star anise nanoemulsion, where highest toxicity was observed. It produced a relatively high rate of abnormalities as well. The detected abnormalities included pupal-adult intermediates (PA) or flies that were dead at the point of transition between the pupae and adult life stages (5.67%); malformed adults (MA) or deformed adults flies (29.33%); Clove and turmeric essential oils, respectively, rated second and third for toxicity after mosquito adult exposure with more moderate pupal development impact and reduced abnormalities. The synthetic insecticide α -cypermethrin showed 60.33% normal adult emergence indicating a minor higher effect than that of star anise oil. The NP9 negative control, developed successfully in 100% of cases (Table 2), indicating no toxic or malformation effects. The morphophysiological defects (PA, MA and NA) of the *Musca domestica* pupae and adults were shown in Figure 1.

Table 1. Effects of Volatile Compounds from Ten Medicinal Plants at Concentrations of 1%, 5%, and 10% on the Mortality of *M. domestica* Pupae After 10 Days of Exposure

Treatment	Pupal mortality (%) \pm SE at each concentration		
	1%	5%	10%
Star anise nanoemulsion	6.33 \pm 0.58	17.33 \pm 2.00	35.00 \pm 6.35
Clove nanoemulsion	2.67 \pm 0.58	12.33 \pm 2.52	17.00 \pm 14.73
Turmeric nanoemulsion	1.33 \pm 1.53	10.67 \pm 4.51	16.00 \pm 5.20
α -cypermethrin (positive control)	6.67 \pm 3.06	18.00 \pm 4.58	39.67 \pm 3.00
NP9 (negative control)	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

Table 2. Toxicity of essential oils from medicinal plants at 1%, 5%, and 10% concentrations on mortality and morphophysiological abnormalities of *M. domestica* pupae

Treatment	Concentration	% Mortality and Physiological Abnormality		
		PA	MA	NA
Star anise nanoemulsion	1%	2.67	3.66	93.67
	5%	3.33	14	82.67
	10%	5.67	29.33	65.00
Clove nanoemulsion	1%	0.67	2.00	97.33
	5%	2.33	10.00	87.67
	10%	3.67	13.33	83.00
Turmeric nanoemulsion	1%	0.33	1.00	98.67
	5%	0.33	10.34	89.33
	10%	1.00	15.00	84.00
α -cypermethrin (positive control)	1%	3.67	3.00	93.33
	5%	17.34	0.66	82.00
	10%	23.33	16.34	60.33
NP9 (negative control)	1%	0.00	0.00	100.00
	5%	0.00	0.00	100.00
	10%	0.00	0.00	100.00

**Figure 1.** Morphophysiological abnormalities in *M. domestica* pupae and adults (A: Malformed pupal-adult intermediate; B: Malformed adult; C: Normal adult)

Lethal concentration values

According to Table 3, star anise essential oil had a LC₅₀ value of 11.89% and a LC₉₀ value of 25.71% for the fourth instar larvae of housefly (*M. domestica*), while synthetic insecticide α -cypermethrin recorded LC₅₀ value of 11.09% and LC₉₀ value of 24.12%.

Table 3. Lethal concentration for *M. domestica* pupae mortality after 10 days of exposure

Treatment	Lethal Concentration (%)	
	LC ₅₀	LC ₉₀
Star anise nanoemulsion	11.89	25.71
Clove nanoemulsion	34.32	52.68
Turmeric nanoemulsion	39.27	57.80
α -cypermethrin (positive control)	11.09	24.12
NP9 (negative control)	N/A	N/A

Lethal Concentration 50 (LC₅₀) refers to the concentration of a substance that causes 50% mortality in *M. domestica* pupae.

Lethal Concentration 90 (LC₉₀) refers to the concentration of a substance that causes 90% mortality in *M. domestica* pupae.

Not Available (N/A) indicates that statistical analysis could not be performed.

Discussion

In this research based on volatile oils of plants, trans anethole which is the main component of star anise exhibited potent. It was found to have a half-lethal concentration (LC₅₀) of 11.89%. (Pour *et al.*, 2022). They have also been shown to inhibit acetylcholinesterase (AChE) activity (Wang *et al.*, 2021), impair digestion, and induce physiochemical abnormalities. In Mediterranean flour moth larvae, for example, *trans*-anethole disrupted acetylcholine transmission and altered the antioxidant defense system (Shahriar *et al.*, 2018). This biological activity indicates that trans-anethole works like commercial insecticides but without the environmental risks.

The use of synthetic pyrethroid-based pest control agents resulted in resistant insect populations, environmental pollution, and negative effects on human health (Abbas and Hafez, 2023). For example, the chemical agents containing pyrethroids which can be harmful to human beings, contaminate ecosystems and cause resistance in insect species (Bass and Field, 2011). Moreover, houseflies can rapidly develop high resistance to cypermethrin (Hafez *et al.*, 2021). The use of synthetic insecticides was initially very effective, with control rates of up to 90% reported (Dong *et al.*, 2007). Nevertheless, the effectiveness of chemicals like cypermethrin has gradually decreased, and their action became slower mainly because of insect population resistance (Zhang *et*

al., 2022). Thus, plant-derived insecticides provide a potential alternative over conventional chemical agents, having distinct advantages. They also tend to avoid issues with resistance and are safe for non-targets and beneficial organisms (Giunti *et al.*, 2022).

Based on these findings, it can be concluded that our compound derived from the plant showed significant potential in improving control of housefly pupae and could therefore lead to natural compounds that are effective insecticides able to reduce or even replace synthetic chemicals, in full agreement with a study by Mahanta and Khanikor (2021).

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Conflict of interest

The authors declare no conflict of interest.

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