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## Larvicidal efficacy and morphological abnormalities induced by plant essential oils against housefly (*Musca domestica* L.)

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Passara, H.<sup>1</sup>, Sittichok, S.<sup>2</sup>, Mounthipmalai, T.<sup>1</sup>, Laosinwattana, C. <sup>1</sup>, Thongsaiklaing, M.<sup>3</sup>, Murata, K.<sup>4</sup> and Soonwera, M.<sup>1\*</sup>

<sup>1</sup>Office of Administrative Interdisciplinary Program on Agricultural Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand; <sup>2</sup>School of Agriculture and Cooperatives, Sukhothai Thammathirat Open University, Nonthaburi, 11120, Thailand; <sup>3</sup>Faculty of Agriculture, Princess of Naradhiwas University, Narathiwat, 10540, Thailand; <sup>4</sup>School of Agriculture, Tokai University, Kumamoto, 862-8652, Japan.

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**Abstract** The results showed that the lowest complete pupation rate was 43.67%, indicating a significant inhibition of normal metamorphosis. Additionally, 56.33% of third-instar larvae were classified as malformed larvae (ML), dying before pupation and exhibiting severe morphological and physiological abnormalities. These findings suggest that the star anise nanoemulsion interferes with key developmental pathways, preventing larvae from reaching the pupal stage. Due to its high effectiveness and plant-based origin, this nanoformulation offers a promising, environmentally friendly alternative to conventional chemical insecticides. It has potential for further development into a natural larvicidal agent for use in integrated pest management (IPM) strategies targeting housefly populations. Future research should explore the underlying mechanisms of action and assess the efficacy and safety of this nanoemulsion under real-world conditions to support its application in sustainable vector control.

**Keywords:** Botanical insecticide, Housefly, Larvicidal, Morphological abnormalities, Plant essential oils

### Introduction

Thailand is located in the tropic zone, which have a climate favorable for breeding of many disease vector animals, especially housefly (*Musca domestica* L.) (Geden *et al.*, 2021). This species, belonging to order Diptera and family Muscidae, is identified as an important vector found in all the provinces of Thailand (Sukontason *et al.*, 2000). It is a significant concern both for public health and for livestock (Khamesipour *et al.*, 2018). Houseflies land on humans

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\*Corresponding Author: Soonwera, M.; Email: [mayura.so@kmitl.ac.th](mailto:mayura.so@kmitl.ac.th)

and animals more frequently than any other species of fly, which makes them effective vectors for bacteria, viruses, protozoa and various pathogens to human beings and animals due to their indiscriminate contact with surfaces or materials (Sommer *et al.*, 2024). These pathogens in cling to the body, legs, wings and mouth parts of the fly as well potential for further residence within their internal systems (Cook *et al.*, 1999). Consequently, house flies are recognized vectors of many diseases including food poisoning, diarrhoea, cholera, dysentery typhus, typhoid fever and anthrax. In addition, they serve as mechanical transmission vehicles for the eggs of many parasitic worms that infect humans (Awache and Farouk, 2016). As found by Maipanich *et al.* (2010), a study of 51 tourist locations in the 22 provinces of Thailand found house flies with eggs and larvae from 12 species of parasitic worms. They include roundworms, whipworms, hookworms, pinworms, pork tapeworms, beef tapeworms, liver flukes, and intestinal and lung flukes. Also, housefly larvae can lead to myiasis (infestation of living tissue or internal organs by fly larvae) in humans and animals (Dogra and Mahajan, 2010). Apart from disease transmission, house flies also contribute to a considerable nuisance by walking on human bodies—faces, arms, legs—and food that we have while eating. The foul-smelling breeding grounds are also detrimental to the aesthetic appeal of the the surrounding area (Hamidou Leyo *et al.*, 2021).

Thailand has always been a fascinating destination for tourists, both domestic and international tourists, steadily increasing every year due to Thailand's tourism promotion policies. As a result, accommodations, restaurants, and recreational facilities are on the rise (Barreiro *et al.*, 2013). Nonetheless, this growth has also been accompanied by an increase in waste generation. The uncontrolled disposal of waste potentially leads to the establishment of fly breeding grounds (Ileke *et al.*, 2020). Not only do these flies eat waste, they also interrupt tourists' leisure and eating activities. FAO read more Factsheet caption: House fly as an indicator of poor sanitation. It is alarming that tourists in these areas may become infected with pathogenic organisms if these flies carry such pathogens. Transmitters as these have the potential to undermine tourism industry and leading towards impact on economy and bad reputation for the industry (Shahanaz *et al.*, 2025).

Study of larvicidal activity and morphological abnormalities in larvae of houseflies (*M. domestica* L.) using essential oils from selected medicinal plants. The plant used in the experiment were herbal for which it is known to be non-toxic in humans, animals and environment.

## **Materials and methods**

### ***Maintenance and rearing of *Musca domestica****

Hand-collected mature *M. domestica* L. were obtained from a market in Bang Phli (latitude: 13.6050453; longitude: 100.6925656) and maintained in an enclosed cube of size 300 × 300 × 300 mm<sup>3</sup> under indoor controlled conditions (25±2°C) at the Entomology and Environmental Science Laboratory, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok during the study period. Adult flies fed on cotton pads saturated with a 10% solution of sugar and a mixture of powdered milk and dog kibble. Coconut fibre (sterilised before delivery by the producer) was used without further treatments. Mackerel (300 g in total) was added into the sterilised coconut substrate inside a plastic container acting as both a deposition site for eggs and nutrient source for developing larvae. Experimental larvae: Selection of third-instar larvae harvested three days post-hatching.

### ***Formulation of nanoemulsified plant essential oils***

Nanoemulsions were prepared using a mixture of emulsifying agents—Nonyl Phenol Ethoxylate (NP9). The essential oils used in the formulations exhibited remarkable miscibility without evidence of phase separation or sedimentation. The mixed solutions were then characterized for droplet size and surface charge using a particle characterization instrument. Particle analyser (NanoPlus Zeta, Otsuka Electronic Co., Ltd., Osaka, Japan) was used to measure droplet size and Zeta potential. They varied from 24.1 to 56.8 nanometers, and all formulations were below the nanoscale (i.e., <100 nm). The zeta potential measurements ranged from -11.23 to -9.07 mV, indicating moderate colloidal stability.

### ***Mortality assay***

The dipping method was then used based on the work of Sayampol et al. (1993), under a completely randomised design (CRD) with three replications per treatment. Ten 3rd instar housefly larvae were pooled for each replicate. The larvae were immersed in 100 µL of herbal essential oil diluted in NP9 for 10 s and transferred into plastic boxes containing food appropriate for the larval stage and moist coconut husk used as a rearing medium. We monitored larval mortality at 10, 30 and 60 min as well as at 24 h post-treatment. NP9 was used as negative control and  $\alpha$ -cypermethrin as positive control and the same procedures regarding essential oil treatments were applied in repeating the experiment for validation. The study further tested the toxicity of essential oils against larval

stages of housefly. Morphological abnormalities were evaluated and classified according to Khalil *et al.* (2010), as well as Malformed larvae (ML)–larvae that arrested and did not complete metamorphosis to form a pupa.

### ***Statistical data analysis***

The average percentages of larval mortality were calculated. Probit analysis was used to calculate the lethal time required to kill 50% (LT<sub>50</sub>) and 90% (LT<sub>90</sub>) of the larvae. Duncan's Multiple Range test (DMRT) was used to compare the treatment groups and determine if statistical differences exist among their mean values.

## **Results**

### ***Toxic effects of medicinal plant essential oils on mortality of Third-Instar Housefly Larvae (*Musca domestica*)***

Among the three medicinal plants, however, a 1% nanoemulsion of star anise essential oil showed the highest larvicidal activity with 56.33% mortality at post-exposure time of 1,440 minutes against third instar larvae (*M. domestica*). In comparison, the synthetic insecticide  $\alpha$ -cypermethrin (positive control) had a mortality rate of 34.00% only. Clove and turmeric nanoemulsions exhibited lower maximum mortality rates of 20.67% and 12.56%, respectively. Furthermore, the NP9 solution applied as the negative control had no effect on third-instar housefly larvae mortality (Table 1).

**Table 1.** Effect of 1% concentration of nanoemulsion from medicinal plants on the mortality of third-instar housefly larvae (*M. domestica*) at 10, 30, 60, and 1,440 minutes after exposure

Type of substance	Percentage mortality rate (mean $\pm$ SD)			
	10 min	30 min	60 min	1440 min
Star anise nanoemulsion	36.33 $\pm$ 28.02	40.67 $\pm$ 9.07	50.33 $\pm$ 29.14	56.33 $\pm$ 7.12
Turmeric nanoemulsion	2.00 $\pm$ 21.82	5.67 $\pm$ 9.32	8.00 $\pm$ 7.34	12.56 $\pm$ 9.12
Clove nanoemulsion	8.00 $\pm$ 5.00	12.67 $\pm$ 8.50	15.00 $\pm$ 7.21	20.67 $\pm$ 8.39
NP9 (negative control)	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
$\alpha$ -cypermethrin (positive control)	23.67 $\pm$ 4.51	28.67 $\pm$ 5.13	31.00 $\pm$ 5.00	34.00 $\pm$ 4.58

The lethal time for 50% (LT<sub>50</sub>) and 90% (LT<sub>90</sub>) of third-instar housefly larvae (*M. domestica*) was determined with respect to medicinal plant essential

oils containing LC<sub>50</sub> of 1% essential oil indicated that essential oil a star anise had LT<sub>50</sub> = 1.34 hours, LT<sub>90</sub> = 2.66 hours. In contrast, the synthetic insecticide and positive control  $\alpha$ -cypermethrin had a LT<sub>50</sub> of 1.86 hours and an LT<sub>90</sub> of 3.23 hours (Table 2).

**Table 2.** Lethal Time for housefly larvae (*M. domestica*)

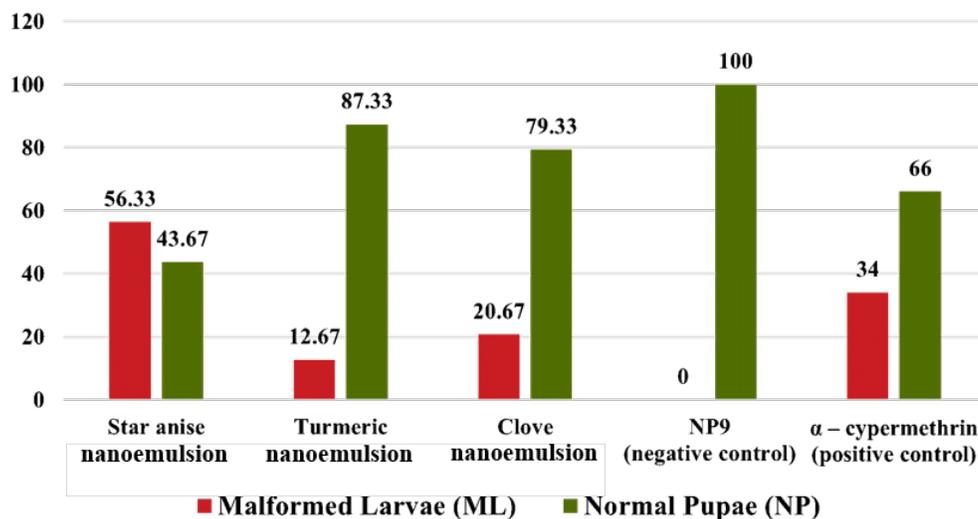
Type of substance	Lethal time (hour)	
	LT <sub>50</sub>	LT <sub>90</sub>
Star anise nanoemulsion	1.34	2.66
Turmeric nanoemulsion	2.38	6.03
Clove nanoemulsion	2.12	4.25
NP9 (negative control)	N/A	N/A
$\alpha$ -cypermethrin (positive control)	1.86	3.23

Lethal Time 50 (LT<sub>50</sub>) refers to the time required to cause 50% mortality in third-instar housefly larvae (*M. domestica*).

Not Available (N/A) indicates that the statistical value could not be analysed.

***Larvicidal and morphophysiological impacts of medicinal plant volatiles on third-instar housefly larvae (*M. domestica*)***

*M. domestica* mortalities and morphophysiological abnormalities observed when exposed to a nanoemulsified extract from three medicinal plants at 1% concentration (Figure 1). The three nanoemulsions exhibited distinct effects on larval development and survival. Star anise essential oil nanoemulsion was the most toxic among them, with only 43.67% complete pupation and 56.33% malformed larvae (ML)—larvae that died without successful pupation. The adult emergence rate was 79.33% (ML=20.67%) for the clove nanoemulsion and 87.33% (ML=12.67%) for the turmeric nanoemulsion, respectively. In comparison to this, the synthetic insecticide  $\alpha$ -cypermethrin, which was used as a positive control in this study, produced 34.00% malformed larvae and 66.00% development into pupae from the examined larvae.  $\alpha$ -cypermethrin had a smaller effect on the insect's development than the herbal preparations. The negative control (NP9 solution) did not have any adverse effects with 100% of the larvae developing into pupae.



**Figure 1.** Toxicity of essential oils from medicinal Plants at 1% concentration on mortality and morphological and physiological abnormalities in housefly larvae (*M. domestica*).

## Discussion

Botanical-derived insect pest control is a proven alternative to combat flies and their associated vector-driven diseases with more safety. The study is based on the strong larvicidal property of star anise essential oil and its combination with another essential against *M. domestica* (Sripongpun, 2008). Nonetheless, they should be the first line of defence along with preventive strategies and environmental management. Synthetic insecticides commonly used in agriculture are associated with environmental damage, human health risk, and development of resistance (Pathak *et al.*, 2022). Because of their documented bioactivity, plant-based essential oils and their active constituents may serve as alternative candidates to develop newer effective larval fly control formulations (Soonwera, 2015). This works demonstrates that nanoemulsion formulations from plant essential oils show good larvicidal activity on fly maggots. A 1% solution of star anise was particularly effective, killing more larvae than the traditional chemical insecticide cypermethrin. In general, combinations of two essential oils show more rapid toxic effects ( $LT_{50}$  and  $LT_{90}$  values) than  $\alpha$ -cypermethrin. These results are consistent with previous studies in which a combination of 0.5% star anise and 0.5% geranial achieved full paralysis and death of houseflies within a  $LT_{50}$  of only 6 minutes (Aungtikun *et al.*, 2021). Likewise, combinations like 1% lemongrass essential oil with 1% *trans*-anethole, and 1% star anise mixed with 1% geranial showed outstanding egg-

killing efficacy, between (94%) to (96%). (Passara *et al.*, 2024). In addition, star anise has shown a high level of toxicity against the immatures and adult forms of the rust grain beetle (Wang *et al.*, 2021), served as a repellent against houseflies (Sinthusiri and Soonwera, 2014) and was lethal to the cherry vinegar fly (Kim *et al.*, 2016). It represented total obliteration ovicidal activity on mosquito eggs with LC<sub>50</sub> value were 1.0% and 1.4% (Puwanard and Soonwera, 2022).

It is well known that the synthetic pyrethroid  $\alpha$ -cypermethrin (Yao *et al.*, 2019) disrupts normal function of nervous system by hindering sodium channels in nerve cell membrane thereby inducing neurotoxicity (Singh *et al.*, 2012). This study revealed that  $\alpha$ -cypermethrin was less toxic to housefly larvae compared with those using nanoemulsions created from star anise essential oil when blend with other essential oil. Insects developed resistance over the years due to its persistent use, making it difficult to manage pests (Duhan *et al.*, 2017), and leading to natural habitat contamination. Consequently, plant-based insecticides have become a top option since they not only do no harm to humans and animals but also do not harm the ecology.

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### **Conflicts of interest**

The authors declare that there are no conflicts of interest.

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