
Insecticidal efficiency of plant essential oil nanoemulsion formulas against *Spodoptera exigua*

Passara, H.¹, Pumnuan, J.¹, Thongsaiklaing, T.² and Thipmanee, K.^{1*}

¹School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok, 10520, Thailand; ²Faculty of Agriculture, Princess of Naradhiwas University, Narathiwat, 96000, Thailand.

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Abstract The findings revealed that the nEO formula of *I. verum* and *C. longa* demonstrated optimal effectiveness, with a concentration of 0.35% resulting in the highest mortality rate of 43.3%, 100% antifeedant effect and 85.0% growth inhibition activities in pupae. Additionally, a concentration of 0.25% led to 100% growth inhibition activities in adults. Consequently, the nEO formulas of *I. verum* and *C. longa* promise for the future development of botanical insecticides targeting the control of *S. exigua*.

Keywords: Antifeedant, Growth inhibition, Mortality, Nanoemulsion, *Spodoptera exigua*

Introduction

Spodoptera exigua, a prevalent polyphagous pest within the Lepidoptera family, poses a significant threat to numerous crops (Han *et al.*, 2014). The larval stage of this insect is particularly destructive, causing substantial damage to leaves, fruits, and flowers, ultimately leading to decreased crop productivity. Notably, it has been identified as one of the most resistant insect pests to chemical insecticides in Thailand, with the use of such chemicals posing risks to beneficial insects and leaving residues in both production and the environment. Biological control methods have proven to be less effective in managing *S. exigua*. Consequently, the adoption of organic alternatives, such as essential oils and crude extracts, has emerged as a highly effective and environmentally safe strategy for insect pest control.

In the realm of integrated pest management, botanical insecticides stand out as a particularly effective approach to control various insect pests, offering antifeedant properties as well as inhibiting growth and development (Wang *et al.*, 2016). Their utilization aids in reducing the reliance on chemical insecticides

* **Corresponding Author:** Thipmanee, K.; **Email:** ammorn.in@kmitl.ac.th

within integrated pest management programs (Gregg *et al.*, 2018). The careful selection of botanical insecticides not only proves highly effective but also aligns with environmentally friendly practices, serving as a viable replacement or reduction of chemical insecticides that can be detrimental to agricultural production. Presently, the use of botanical insecticides in controlling agricultural pests has gained popularity among farmers (Gerken *et al.*, 2022).

Previous studies highlight the notable insecticidal effects of specific botanicals, such as *I. verum*, *C. longa*, and *S. aromaticum*. *I. verum*, for instance, has demonstrated effectiveness in insect pest control, with its chemical compound trans-anethole exhibiting high efficacy on acetylcholinesterase activity in insect pests (Peter *et al.*, 2022; Cruz *et al.*, 2013). Similarly, *C. longa*, through its component ar-turmerone, has exhibited high mortality rates in the larvae stage of *Plutella xylostella* and *Spodoptera litura*, affecting acetylcholine esterase and inducing larval mortality (Lee *et al.*, 2001; Rao *et al.*, 2022). *S. aromaticum*, containing eugenol, has displayed potent effects on nerve systems and acetylcholinesterase inhibition, providing effective control over insect pests (Regnault *et al.*, 2012). Furthermore, *S. aromaticum* has demonstrated both insecticidal and repellent effects on various insect pests (Chaieb *et al.*, 2007).

Essential oil nanoemulsions (nEOs) represent essential oils encapsulated within materials, with a nanometer range typically spanning 1-100 nm (Kumar and Kumari, 2019). Studies on essential oils have revealed that the oil phase, when released from the core, exhibits more effective properties than the oil alone (Nasr *et al.*, 2020). Nanoemulsions consist of three phases, namely oil, surfactant, and water. Surfactants, serving as emulsifiers in nanoemulsions, come in cationic, anionic, amphoteric, and nonionic forms (Devarajan and Ravichandran, 2011). The hydrophile-lipophile balance (HLB) range of 10-16 is commonly employed in oil-in-water emulsions for insecticides in agriculture, ensuring kinetically stable nanoemulsions. The addition of surfactants can alter the electrostatic charge within the nanoemulsion, leading to reduced aggregation (Feng *et al.*, 2016).

The preparation of nanoemulsions can be accomplished through the aqueous titration method, employing surfactants as emulsifiers to control droplet size distribution (Ariyaprakai, 2017). This method is cost-effective compared to alternative approaches and offers simplicity in mixing essential oils, surfactants, and water (McClements and Rao, 2011). Nanoemulsions have the capability to coat the cuticles of insect pests, enhancing the absorption of active ingredients. This coating disrupts the wax cuticular layer in insect pests, causing dehydration and ultimately leading to mortality (Omar and Kordali, 2019).

The objective was to focus on testing the efficacy of a blend of essential oils namely, nanoemulsions from *I. verum*, *C. longa*, *S. aromaticum*, and main

chemical compounds in the form of insecticides, feeding deterrents and growth inhibitors.

Materials and methods

The procedural approach was undertaken to delineate the procedures involved in conducting tests on the *S. exigua* culture. This encompassed the preparation of plant essential oil nanoemulsion and the formulation of key chemical compounds in the form of equations. The leaf dipping method was employed, and the experiment focused on assessing mortality, antifeedant, and growth inhibition effects on *S. exigua* under laboratory conditions.

***Spodoptera exigua* cultivation**

Samples of *S. exigua* were gathered from *Brassica rapa* and *Brassica oleracea* plots located in Nakhon Pathom, Chachoengsao, and Nonthaburi, Thailand. Subsequently, they were raised in insect boxes under ambient conditions at a temperature of 25 ± 2 °C and a 12:12 light-dark cycle. This cultivation was conducted at the Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL) in Bangkok, Thailand. The larvae were provided with fresh *Brassica rapa* leaves every 1-3 days, while the adult insects were given honey, with changes made every 3 days.

Cultivation of Brassica rapa

Brassica rapa plants were grown without the use of insecticides as a source of food for insect testing at the previously mentioned location. Following the planting process, a fertilizer with a formula of 46-0-0 was applied, and the plants were watered twice daily.

Mortality test under laboratory conditions

The experiment employed a no-choice test, wherein five leaves of *Brassica rapa* were prepared and immersed in various concentrations of plant nanoemulsions and key chemical compounds, ranging from 0.00% (surfactant, control) to 0.35%, for one minute. Subsequently, the treated leaves were air-dried at room temperature for 15 minutes. Ten second-stage larvae of *S. exigua* were then placed on these *Brassica rapa* leaves and enclosed in boxes (with three

replications). After a 24-hour period, the observed mortality rates were calculated and compared to the control group using Abbott's formula (Abbott, 1925).

Antifeedant effect test under laboratory conditions

In a no-choice test, the antifeedant effect resulting from the application of plant nanoemulsions and primary chemical compounds was assessed using the methods described above. *Brassica rapa* leaves, each with a 3 cm diameter, were immersed in these formulations of plant nanoemulsions and main chemical compounds at concentrations varying from 0.00% (surfactant, control) to 0.35% for one minute. The treated leaves were then air-dried at room temperature for 15 minutes. Subsequently, ten second-stage larvae of *S. exigua* were placed on the *Brassica rapa* leaves and enclosed in boxes (with three replications). The antifeedant effect was observed after 24 hours, and the consumed area was measured and compared with the control group. The percentage of antifeedant effect was calculated and expressed as the antifeedant index (AFI), as indicated by the formula:

$$\text{AFI} = [\%T / (\%T + \%C)] \times 100$$

Where C and T represent the areas consumed on controlled and treated leaves, respectively (Escoubas *et al.*, 1992).

Growth inhibition test under laboratory conditions

The growth inhibition test was conducted in a no-choice setting, employing the methods outlined earlier to evaluate the growth inhibitory effects resulting from the application of plant nanoemulsions and primary chemical compounds. *Brassica rapa* leaves, each with a 3 cm diameter, were immersed in varying concentrations of these formulations, ranging from 0.00% (surfactant, control) to 0.35%, for one minute. Following immersion, the leaves were air-dried at room temperature for 15 minutes. Each concentration was positioned on the opposite side of the control in the experimental box. Subsequently, ten second-stage larvae of *S. exigua* were placed on these *Brassica rapa* leaves, and the larvae were maintained in the box with daily food changes (with three replications). The developmental progress from larvae to pupa and adult stages was observed and compared with the control group. The recorded data included the number of pupae developed from the larval stage and the number of adults emerging from the pupal stage.

Statistical analysis

Abbott's formula was applied to obtain *Spodoptera exigua* mortality rate. The experiment was performed in a completely randomized design (CRD) with three replicates per treatment. The obtained data were analyzed by ANOVA program.

Results

Preparation of nanoemulsion formulas with plant essential oil and main chemical compounds

For the preparation of nanoemulsions, specific formulas were chosen involving plant essential oils and main chemical compounds. Two surfactants, Tween 20 and NP 9, were employed for emulsion creation. It was observed that Tween 20 and NP 9, with hydrophile-lipophile balance (HLB) values of 16.7 and 12.9 respectively, exhibited no precipitation and high solubility. This was evident in formulations such as the mixture of *I. verum* essential oil with Tween 20 at a ratio of 1:4 combined with *C. longa* essential oil with NP 9 at a ratio of 1:2 in a 1:1 ratio. Additionally, the formula of *I. verum* essential oil with Tween 20 at a ratio of 1:4 blended with *S. aromaticum* essential oil with NP 9 and Tween 20 at a ratio of 1:2.5:3 at a 1:1 ratio displayed similar characteristics. The main chemical compounds in these formulations used surfactant ratios analogous to their respective plant essential oils. For instance, the trans-anethole emulsion with Tween 20 at a ratio of 1:2.5 mixed with ar-turmerone emulsion with NP9 at a ratio of 1:2 in a 1:1 ratio.

These formulations demonstrated high solubility, avoiding precipitation. Consequently, their emulsions underwent particle size and zeta potential measurements using a particle analyzer. The resulting particle sizes for *I. verum* with *C. longa*, *I. verum* with *S. aromaticum*, trans-anethole with ar-turmerone, and trans-anethole with eugenol nanoemulsions were 15.5, 15.8, 13.0, and 27.6 nm, respectively. The corresponding zeta potentials for these formulations were -19.09, -22.18, -21.82, and -18.39 mV, respectively. This investigation indicates that the nanoemulsion formulas achieved small particle sizes below 100 nm.

Mortality impact of nanoemulsion formulas with essential oils on *S. exigua*

The most pronounced mortality activity against *S. exigua* was observed in the nanoemulsion formula combining *I. verum* with *C. longa* essential oil, registering a mortality rate of 43.3% at a concentration of 0.35%. Conversely,

the nanoemulsion formula featuring trans-anethole with ar-turmerone essential oil, acting as the primary chemical compound (nMC) in these plant nanoemulsions, exhibited the highest mortality effect at 41.7%, observed at the 0.35% concentration, as detailed in Table 1.

The calculated LC50 and LC90 values for the *I. verum* with *C. longa* essential oil nanoemulsion formulas were 0.319 and 0.429, respectively. In comparison, the trans-anethole with ar-turmerone essential oil nanoemulsion formula yielded LC50 and LC90 values of 0.331 and 0.440, respectively, as outlined in Table 1.

Table 1. The Mortality percentage of *Spodoptera exigua* caused by various nanoemulsion formulas

Nanoemulsion formulas	Average mortality (%)								
	Concentrations (%)								
	0.00	0.10	0.15	0.20	0.25	0.30	0.35	LC ₅	LC ₉
<i>I. verum: C. longa</i>	0.0±0.0 ^{0c}	0.0±0.0 ^{Ae}	0.0±0.0 ^{Ae}	16.7±0.5 ^{Ad}	21.7±0.4 ^{Ae}	25.0±0.6 ^{Ab}	43.3±0.5 ^{Aa}	⁰ 0.31 9	⁰ 0.42 9
<i>I. verum: S. aromaticum</i>	0.0±0.0 ^{0c}	0.0±0.0 ^{Ae}	0.0±0.0 ^{Ae}	5.0±0.6 ^{Cd}	13.3±0.5 ^{Ce}	18.3±0.4 ^{Cb}	35.0±0.6 ^{Ca}	0.36 3	0.47 5
Trans-anethole: Ar-turmerone	0.0±0.0 ^{0c}	0.0±0.0 ^{Ae}	0.0±0.0 ^{Ae}	13.3±0.5 ^{Bd}	18.3±0.4 ^{Bc}	21.7±0.8 ^{Bb}	41.7±0.4 ^{Ba}	0.33 1	0.44 0
Trans-anethole: Eugenol	0.0±0.0 ^{0c}	0.0±0.0 ^{Ae}	0.0±0.0 ^{Ae}	3.3±0.5 ^{Dd}	6.7±0.5 ^{Dc}	15.0±0.6 ^{Db}	31.7±0.4 ^{Da}	0.37 1	0.47 0

Means in column followed by the same uppercase letter and means in a row followed by the same lowercase letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

Antifeedant impact of nanoemulsion formulas with essential oils on S. exigua

The examination of antifeedant effects revealed that the maximum antifeedant rate, reaching 100%, was observed with the 0.35% concentration of *I. verum: C. longa* essential oil nanoemulsion formulas against *S. exigua*. Conversely, the trans-anethole with ar-turmerone essential oil nanoemulsion formula exhibited the highest antifeedant rate at 87.6% with the 0.35% concentration, as indicated in Table 2.

Growth inhibition of nanoemulsion formulas with essential oils on S. exigua

The nanoemulsion formulas combining *I. verum* with *C. longa* essential oil, at a concentration of 0.35%, exhibited the most significant inhibition of growth, with 85% inhibition for pupal development from the larval stage and

100% inhibition for adult development from the pupal stage in *S. exigua*. Notably, all essential oil nanoemulsion formulas, across various concentrations, demonstrated greater effectiveness in the pupal stage as compared to the adult stage of the insect, as detailed in Table 3.

Table 2. The antifeedant percentage of various nanoemulsion formulas against *Spodoptera exigua*

Nanoemulsion formulas	Average of antifeedant rate (%)						
	Concentrations (%)						
	0.00	0.10	0.15	0.20	0.25	0.30	0.35
<i>I. verum: C. longa</i>	0.0±0.0 _g	28.4±12.6 _{Af}	34.8±14.7 ^A _e	43.0±18.0 ^A _d	63.6±26.2 ^A _c	80.8±33.9 ^A _b	100.0±0.0 ^A _a
<i>I. verum: S. aromaticum</i>	0.0±0.0 _g	18.4±9.7 ^{Cf} _e	29.8±14.9 ^C _e	33.8±15.3 ^C _d	50.6±21.0 ^C _c	75.2±31.6 ^B _b	86.8±39.0 ^B _a
Trans-anethole:Ar-turmerone	0.0±0.0 _g	21.0±9.7 ^{Bf} _e	30.6±13.1 ^B _e	36.4±15.2 ^B _d	55.0±25.2 ^B _c	76.6±31.4 ^B _b	87.6±36.0 ^B _a
Trans-anethole:Eugenol	0.0±0.0 _g	16.8±11.0 _{Df}	21.6±11.4 ^D _e	29.2±15.1 ^D _d	49.4±21.0 ^D _c	70.6±30.0 ^C _b	82.4±34.7 ^C _a

Means in column followed by the same uppercase letter and means in a row followed by the same lowercase letter are not significantly different (P < 0.05) according to Duncan's multiple range test.

Table 3. The growth inhibition percentage of various nanoemulsion formulas against pupa and adult stage of *Spodoptera exigua*

Nanoemulsion formula	Average growth inhibition on pupal stage (%)						
	Concentrations (%)						
	0.00	0.10	0.15	0.20	0.25	0.30	0.35
Pupal stage							
<i>I. verum: C. longa</i>	0.0±0.0 ^B	45.0±1.1 ^A _f	58.3±0.5 ^A _e	63.3±1.5 ^A _c	76.7±0.8 ^A _b	81.7±0.8 ^A _a	85.0±0.6 ^{Aa}
<i>I. verum: S. aromaticum</i>	0.0±0.0 ^b	33.3±0.5 ^C _g	48.3±0.4 ^C _f	50.0±0.6 ^C _e	61.7±0.8 ^C _c	68.3±1.2 ^C _b	73.3±1.2 ^{Ca}
Trans-anethole:Ar-turmerone	0.0±0.0 ^c	36.7±0.5 ^B _d	51.7±1.2 ^B _c	58.3±0.8 ^B _c	70.0±1.3 ^B _b	75.0±1.4 ^B _a	76.7±1.5 ^{Ba}
Trans-anethole:Eugenol	0.0±0.0 ^c	28.3±1.2 ^D _d	41.7±0.8 ^D _c	45.0±0.6 ^D _c	55.0±0.6 ^D _b	63.3±0.8 ^D _a	68.3±0.4 ^{Da}
Adult stage							
<i>I. verum: C. longa</i>	0.0±0.0 _e	76.7±0.5 ^{Ad}	86.7±0.5 ^{Ac}	90.0±0.6 ^A _b	100.0±0.0 ^A _a	100.0±0.0 ^A _a	100.0±0.0 ^A _a
<i>I. verum: S. aromaticum</i>	0.0±0.0 _g	68.3±1.0 ^{Cf}	71.7±0.8 ^{Ce}	76.7±0.5 ^C _d	81.7±0.8 ^{Ce}	90.0±0.6 ^{Bb}	100.0±0.0 ^A _a
Trans-anethole:Ar-turmerone	0.0±0.0 _f	73.3±0.5 ^{Bc}	81.7±0.4 ^{Bd}	85.0±0.6 ^{Bc}	93.3±0.5 ^B _b	100.0±0.0 ^A _a	100.0±0.0 ^A _a
Trans-anethole:Eugenol	0.0±0.0 _f	65.0±0.6 ^D _c	66.7±0.5 ^{De}	70.0±0.6 ^D _d	73.3±0.5 ^D _c	81.7±0.8 ^{Cb}	100.0±0.0 ^A _a

Means in column followed by the same uppercase letter and means in a row followed by the same lowercase letter are not significantly different (P < 0.05) according to Duncan's multiple range test.

Discussion

In this investigation, the reduction of droplet size in nanoemulsion formulas was achieved through the aqueous titration method, employing mixed surfactants and/or co-surfactants in appropriate ratios. The particle size and zeta potential of nanoemulsion formulas, including *I. verum* with *C. longa*, *I. verum* with *S. aromaticum*, and their main compounds, ranged from 15.5 to 27.6 nm and 19.00 to 22.18 mV, respectively. These particle sizes were consistently below 100 nm, and the zeta potential values approached 30 mV, indicating physical stability (Marsalek, 2014). Smaller particle sizes in nanoemulsions contribute to improved homogeneity and increased effectiveness compared to larger sizes.

The study's findings revealed insecticidal properties, encompassing mortality, antifeedant, and growth inhibition, associated with *I. verum*, *C. longa*, and *S. aromaticum* nanoemulsion formulas. The identified main chemical compounds, namely tran-anethole, ar-turmerone, and eugenol for *I. verum*, *C. longa*, and *S. aromaticum*, respectively (Sharafan *et al.*, 2022; Hwang *et al.*, 2016), play pivotal roles as defense mechanisms in plants against insect pests. Previous research has shown that *I. verum* essential oil inhibits the growth of the Gypsy moth (Kostić *et al.*, 2021), and star anise exhibits potent insecticidal effects, causing over 80% mortality in *Drosophila suzukii* (Kim *et al.*, 2016). Tran-anethole from star anise demonstrates efficacy in inhibiting acetylcholinesterase activity in *Crytolestes ferrugineus* (Wang *et al.*, 2021). Additionally, *C. longa* extract exhibits high toxicity against *Bactrocera zonata* (Siddiqi *et al.*, 2011), while ar-turmerone from turmeric inhibits Acetylcholine esterase and Butyrylcholine esterase activities, resulting in larval mortality (Rao *et al.*, 2022). Clove essential oil and its main chemical compound exhibit diverse biological effects (Velluti *et al.*, 2004).

In light of these insights, the *I. verum*, *C. longa*, and *S. aromaticum* nanoemulsion formulas demonstrated potent insecticidal effects on *Spodoptera exigua*. Future investigations may focus on adjusting the ratios of plant essential oil and surfactant formulas to optimize insecticidal efficiency.

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