
Evaluating the insecticidal effect of essential oil nanoemulsion against the cutworm, *Spodoptera litura*

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Abstract The cutworm, *Spodoptera litura* is an important pest of cruciferous vegetables and it causes economic impact worldwide. The efficiency of turmeric and star anise essential oil nanoemulsions (nEOs) against the cutworm, including mortality, antifeedant, and growth inhibition effects was investigated. Essential oil nanoemulsions (nEOs) were prepared by mixing the essential oils with surfactants and co-surfactants. The 2nd stage instar larvae of cutworms were tested by leaf dipping method and were observed after 24 hours. The results showed that the two nEOs, which were turmeric and star anise, performing the mortality, and growth development effect on *Spodoptera litura* as well as having antifeedant property. Complete mortality rate was shown at 0.35 % concentration of turmeric and star anise nEOs, when 100% antifeedant effect was found at 0.20% concentrations of nEOs, and 100% growth inhibition was observed at 0.30% concentrations of nEOs. It could be concluded that the nEOs from star anise had antifeedant and insecticide properties.

Keywords: Nanoemulsion, Cutworm, Mortality, Antifeedant effect, Growth inhibition

Introduction

A large quantity of production losses during cultivation due to insect infestation is a main problem of crop production, which can lead to large commercial losses (Haff and Slaughter, 2004, Fornal *et al.*, 2007). Cutworm or *Spodoptera litura* is one of the most common crops pests causing economic impact. Using insecticide to control pests or cutworms is the most popular method (Kranthi *et al.*, 2002) because it is simple to use (Talekar and Shelton, 1993). However, some cutworms have developed resistance to almost all insecticide groups applicable in the field (Chen *et al.*, 2008; Ahmad *et al.*, 2008; Shad *et al.*, 2010) and the insecticide residues are found in plants, soil, and environment, and affect the non-target pests and other livings. Therefore, using plant insecticide instead of chemical insecticide can mitigate those adverse effects.

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Plant essential oils are natural elements distilled from various parts of plant containing pesticide compounds, and it can be used as an alternative to chemical pesticide to mitigate its side effect. Modes of action and properties of plant essential oils, especially poison repellents, antifeedant, and attractants can reduce the amount of insect pests (Elnabawy *et al.*, 2021). Using plant essential oils for pest control causes less harm to mammals and environment (Mossa, 2016), but plant essential oils have some limits, including physical properties, fast degradation and low water solubility. For these reasons, nanoemulsion essential oils are developed to overcome some disadvantages of essential oils by applying nanotechnology approaches.

Nanoemulsion is a class of emulsions with a particle size ranging from 5 to 200 nm (Shah *et al.*, 2011). Nanoemulsions are developed to improve properties of Eos, including physical stability and solubility in the aqueous phase (Joe *et al.*, 2012). Biological properties of nanomaterial play a prominent role in pest management (Abbott, 1925; Finney, 1971; Van Asperen, 1962; Habig *et al.*, 1974). Nanoemulsion formulation offers several advantages, including more effectiveness on pest control, lower toxic effect on non-target organism, and high stability of substance (Anjali *et al.*, 2012).

Therefore, this study aimed to evaluate the efficiency of plants nanoemulsion essential oils in the form of herbicide against cutworm, *Spodoptera litura*, and it can be used as insecticides to reduce the adverse effects of chemical pesticides in the future.

Materials and methods

Cutworm rearing

Cutworms (*Spodoptera litura*) were kept from organic Chinese cabbage (*Brassica chinensis*) plot in Chachoengsao province, Thailand. After that, they were reared and fed by organic Chinese cabbage in insect cage and maintained at room temperature (25 ± 2 °C) and 12:12 light - dark cycle, at the laboratory of Natural Products for Pest Control Research Center (NPCRC), Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand.

Selection of plant essential oils

The insecticidal effect of the essential oil emulsions (EOs) from 12 species of medicinal plants were firstly evaluated against the cutworm to select the most effective essential oil emulsions to be developed into nanoemulsions. The EOs used in this study were purchased from the Thai - China Flavors and

Fragrances Industry Co. Ltd., Thailand, the production of which is in compliance with the principles of hazard analysis and critical control point (HACCP), and Tween – 20 was used as surfactant. The 2 most effective essential oil emulsions were selected for further study.

Preparation of nanoemulsion essential oils

The turmeric and star anise EOs which performed the 2 most effectiveness were diluted with water and polysorbate (Tween - 20, Hydrophilic lipophilic balance (HLB) = 16.7) or nonyl phenol ethoxylate (NP9, HLB = 12.9) as surfactant. In this process, the turmeric essential oil nanoemulsion contained turmeric essential oil and NP9 (surfactant) at a ratio of 1:2. The star anise essential oil nanoemulsion contained Tween - 20 (surfactant) at a ratio of 1:4. The particles sizes of essential oil nanoemulsions (nEOs) were measured with a particle analyzer (NanoPlus Zeta, Otsuka Electronic Co., Ltd., Osaka, Japan). The nEOs particle sizes were found with smaller than 100 nm.

Mortality test

The preliminary efficiency of 12 EOs against the cutworm was firstly evaluated using leaf dipping method. Chinesees cabbage leaves were cut into circle shape with a diameter of 3 cm, then were dipped into EOs at 1 % and 10% concentrations for 1 minute, and dried at room temperature for 15 minutes. Ten 2nd stage larvae of cutworms were released on treated Chinese cabbage leaves in the box and the mortality of cutworm was observed after 24 hours. The most effective EOs in killing cutworms were selected for the next process.

The prepared Chinese cabbage leaves were dipped into various concentrations of nEOs; at 0.00% (water + surfactant), 0.10%, 0.15%, 0.20%, 0.25%, 0.30%, and 0.35% concentrations for 1 minute, and dried at room temperature for 15 minutes. Ten 2nd stage larvae of cutworms were put on treated Chinese cabbage leaves and kept in the box. The mortality was observed after 24 hours and the mortality rates were calculated and compared with the control group using Abbott's formula (Abbott, 1925).

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

Where T is test mortality (%) with EOs, nEOs and C is control mortality (%).

Antifeedant effect test

Antifeedant effect caused by the two selected nEOs (Turmeric and Star anise) was determined by the abovementioned method. The Chinese cabbage leaves with the diameter of 3 cm were dipped in the different concentrations of selected nEOs; at 0.00%, 0.10%, 0.15%, 0.20%, 0.25%, 0.30%, and 0.35%. Ten

2nd stage instar larvae of cutworms were released in the test box. 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 represented by the antifeedant index (AFI) (Escoubas *et al.*, 1992) as

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

where C and T are control and treat leaf area consumed, respectively

Growth inhibited test

The study of growth inhibition effect of turmeric and star anise in the form of nEOs was carried out at 0.00%, 0.10%, 0.15%, 0.20%, 0.25%, 0.30%, and 0.35% concentrations. The Chinese cabbage leaves were dipped into the EOs and nEOs as described above. Ten 2nd stage instar larvae were released in the test box. The development from larvae to pupa and adult were observed. And compared with the control group.

Statistical analysis

The data were analyzed by Abbott's formula, and a completely randomized design method was applied to the experiments with three replicates. The data were analyzed by ANOVA and the diverse treatments were examined by Duncan's multiple range test with a SAS software (SAS Institute, Cary, North Carolina, USA).

Results

In selection of EOs, 100 % mortality of instar larvae cutworms was observed at 1% concentration of turmeric and star anise essential oils (Table 1). The turmeric and star anise EOs had the highest effect against cutworms compared with control group and were selected to develop into nanoemulsion. The particle size in nEOs depends on the amount and type of surfactant. The surfactants in these experiments were Tween - 20 and NP9, the hydrophilic-lipophilic balance (HLB) of which were 16.7 and 12.9, respectively. The turmeric nEOs consisted of 1% turmeric oil and 2% NP9 (surfactant), and the particle size as well as zeta potential were 16.1 nm and - 6.15, respectively. The star anise nEOs in this experiment consisted of 1% star anise oil and 4% Tween - 20 (surfactant), and the particle size and zeta potential were 93.2 nm and 1.27, respectively. From the results, the turmeric and star anise had the highest insecticidal effect against cutworms. Resulting in 100% mortality of instar

larvae was occurred at 0.35% concentrations of turmeric and star anise nEOs after 24 hours (Table 2). At 0.20% concentrations of turmeric and star anise nEOs showed 100% antifeedant effect after 24 hours (Table 3), The interesting result of 100% growth inhibition effect on pupa and adult stages was found at 0.30% concentration (Table 4 and 5). At 0.25% concentrations of turmeric nEOs, the developmental duration of pupa and adult stage were 8.33 ± 0.58 and 7.20 ± 0.42 days, respectively (Table 4 and 5). Where at 0.25% concentrations of star anise nEOs, the developmental duration of pupa and adult stage were 8.33 ± 0.58 and 7.30 ± 0.48 days, respectively (Table 4 and 5). The results implied a decrease in pupa and adult developments compared with control group. As a result, incomplete molting of cutworm was affected by nEOs. However, the effect of turmeric and star anise nEOs on cutworm larvae were not significantly different.

Table 1. Average mortality percentage of the cutworm larvae caused by different plant essential oils (EOs) at 1 and 10 percent concentration after 24h by leaf dipping method

EOs	Average mortality percentage (Mean (%) \pm SD)	
	Concentrations (%)	
	1	10
Siam cardamon	33.33 \pm 0.58 ^{Ac}	33.33 \pm 0.58 ^{Ac}
Eucalyptus	33.33 \pm 0.58 ^{Ac}	33.33 \pm 0.58 ^{Ac}
Holy basil	70.00 \pm 0.00 ^{Bb}	100.00 \pm 0.00 ^{Aa}
Sweet fennel	70.00 \pm 0.00 ^{Bb}	100.00 \pm 0.00 ^{Aa}
Betal vine	20.00 \pm 0.00 ^{Bd}	30.00 \pm 0.00 ^{Ac}
Cinnamon	23.33 \pm 0.58 ^{Ad}	23.33 \pm 0.58 ^{Ad}
Lemon grass	20.00 \pm 0.00 ^{Bd}	33.33 \pm 0.58 ^{Ac}
Star anise	100.00 \pm 0.00 ^{Aa}	100.00 \pm 0.00 ^{Aa}
Clove	33.33 \pm 0.58 ^{Bc}	43.33 \pm 0.58 ^{Ab}
Long pepper	20.00 \pm 0.00 ^{Bd}	33.33 \pm 0.58 ^{Ac}
Black pepper	23.33 \pm 0.58 ^{Bd}	43.33 \pm 0.58 ^{Ab}
Turmeric	100.00 \pm 0.00 ^{Aa}	100.00 \pm 0.00 ^{Aa}
Control	0.00 \pm 0.00 ^{Ae}	0.00 \pm 0.00 ^{Ae}

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

Table 2. Average mortality percentage of the larvae cutworm caused by selected essential oil nanoemulsions (nEOs) after 24h by leaf dipping method

nEOs	Average mortality percentage (Mean (%) \pm SD)							LC50
	Concentrations (%)							
	0.00	0.10	0.15	0.20	0.25	0.30	0.35	
Turmeric	0.00 \pm 0.00 ^{Fa}	0.00 \pm 0.00 ^{Fa}	20.00 \pm 0.00 ^{Ea}	30.00 \pm 0.00 ^{Da}	40.00 \pm 0.00 ^{Ca}	70.00 \pm 0.00 ^{Ba}	100.00 \pm 0.00 ^{Aa}	0.245
Star anise	0.00 \pm 0.00 ^{Ea}	0.00 \pm 0.00 ^{Ea}	20.00 \pm 0.00 ^{Da}	26.67 \pm 0.58 ^{Da}	40.00 \pm 0.00 ^{Ca}	70.00 \pm 0.00 ^{Ba}	100.00 \pm 0.00 ^{Aa}	0.246

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

Table 3. Average leaf feeding area percentage of the larvae cutworm caused by selected essential oil nanoemulsions (nEOs) after 24h by leaf dipping method

nEOs	Average leaf feeding area percentage (Mean (%) \pm SD)						
	Concentrations (%)						
	0.00	0.10	0.15	0.20	0.25	0.30	0.35
Turmeric	60.33 \pm	44.33 \pm	31.67 \pm	0.00 \pm	0.00 \pm	0.00 \pm	0.00 \pm
	2.37 ^{Aa}	1.96 ^{Ba}	2.23 ^{Ca}	0.00 ^{Da}	0.00 ^{Da}	0.00 ^{Da}	0.00 ^{Da}
Star anise	60.33 \pm	45.67 \pm	33.00 \pm	0.00 \pm	0.00 \pm	0.00 \pm	0.00 \pm
	2.37 ^{Aa}	1.81 ^{Ba}	2.64 ^{Ca}	0.00 ^{Da}	0.00 ^{Da}	0.00 ^{Da}	0.00 ^{Da}

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

Table 4. Average growth percentage from larval to pupal stages and duration of pupal stage of cutworm caused by selected essential oil nanoemulsions (nEOs) after 24h by leaf dipping method

nEOs	Average growth percentage from larval to pupal stages (Mean (%) \pm SD)						
	Concentrations (%)						
	0.00	0.10	0.15	0.20	0.25	0.30	0.35
Turmeric	100.00 \pm	90.00 \pm	80.00 \pm	60.00 \pm	30.00 \pm	0.00 \pm	0.00 \pm
	0.00 ^{Aa}	0.00 ^{Ba}	0.00 ^{Ca}	0.00 ^{Da}	0.00 ^{Ea}	0.00 ^{Fa}	0.00 ^{Fa}
Star anise	100.00 \pm	90.00 \pm	80.00+-	60.17 \pm	30.00 \pm	0.00 \pm	0.00 \pm
	0.00 ^{Aa}	0.00 ^{Ba}	0.00 ^{Ca}	0.41 ^{Da}	0.00 ^{Ea}	0.00 ^{Fa}	0.00 ^{Fa}
nEOs	Pupal duration (days) (Mean \pm SD)						
	0.00	0.10	0.15	0.20	0.25	0.30	0.35
	Turmeric	9.40 \pm	9.22 \pm	9.00 \pm	9.00 \pm	8.33 \pm	0.00 \pm
0.52 ^{Aa}		0.44 ^{Aa}	0.00 ^{Aa}	0.00 ^{Aa}	0.58 ^{Ba}	0.00 ^{Ca}	0.00 ^{Ca}
Star anise	9.40 \pm	9.22 \pm	9.00 \pm	9.00 \pm	8.33 \pm	0.00 \pm	0.00 \pm
	0.52 ^{Aa}	0.44 ^{Aa}	0.00 ^{Aa}	0.00 ^{Aa}	0.58 ^{Ba}	0.00 ^{Ca}	0.00 ^{Ca}

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

Table 5. Average growth percentage from pupal to adult stages and adult duration of cutworm caused by selected essential oil nanoemulsions (nEOs) after 24h by leaf dipping method

nEOs	Average growth percentage from pupal to adult stages (Mean (%) \pm SD)						
	Concentrations (%)						
	0.00	0.10	0.15	0.20	0.25	0.30	0.35
Turmeric	100.00 \pm 0.00 ^{Aa}	90.00 \pm 0.00 ^{Ba}	80.00 \pm 0.00 ^{Ca}	50.00 \pm 0.00 ^{Da}	20.00 \pm 0.00 ^{Ea}	0.00 \pm 0.00 ^{Fa}	0.00 \pm 0.00 ^{Fa}
Star anise	100.00 \pm 0.00 ^{Aa}	90.00 \pm 0.00 ^{Ba}	80.00 \pm 0.00 ^{Ca}	50.00 \pm 0.00 ^{Da}	20.00 \pm 0.00 ^{Ea}	0.00 \pm 0.00 ^{Fa}	0.00 \pm 0.00 ^{Fa}
nEOs	Adult duration (days) (Mean \pm SD)						
	0.00	0.10	0.15	0.20	0.25	0.30	0.35
	Turmeric	9.00 \pm 0.00 ^{Aa}	9.00 \pm 0.00 ^{Aa}	9.00 \pm 0.00 ^{Aa}	8.00 \pm 0.00 ^{Ba}	7.20 \pm 0.42 ^{Ca}	0.00 \pm 0.00 ^{Da}
Star anise	9.00 \pm 0.00 ^{Aa}	9.00 \pm 0.00 ^{Aa}	9.00 \pm 0.00 ^{Aa}	8.00 \pm 0.00 ^{Ba}	7.30 \pm 0.48 ^{Ca}	0.00 \pm 0.00 ^{Ea}	0.00 \pm 0.00 ^{Ea}

Mean in a row followed by the same capital letter and means in a column followed by the same country letter are not significant different ($P < 0.05$) according to Duncan's multiple range test

Discussion

The results of this study were in accordance with the following research as Cui *et al.* (2022) found that curcumin had a growth inhibition effect on larvae of *Spodoptera litura* and it could damage the midgut structure of *Spodoptera litura* larvae. *Brassica nigra* and *Curcuma longa* compounds had an effect on *Spodoptera exigua* (Tavares de *et al.*, 2019). Kosti ć *et al.* (2021) found that EOs of anise, dill, and fennel seeds were highly toxic to gypsy moth. Sea fennel EOs has an effect against *Culex quinquefasciatus* and *Spodoptera littoralis* (Pavela *et al.*, 2017). The turmeric extract showed the highest mortality effect on adult of *Tribolium castaneum* (Abida *et al.*, 2010). When the turmeric EO reduced progeny of *Rhyzopertha dominica* and *Sitophilus oryzae* by using contact and fumigant method (Tripathi *et al.*, 2002). The turmeric oil caused low weight larvae, pupae, and adults and it had repellent and growth inhibition effect on red flour beetle (Jilani *et al.*, 1998). Besides, the turmeric showed growth inhibition effect against *Schistocerca gregaria* and *Dysdercus koenigii* (Chowdhury *et al.*, 2000). The growth development of pupal and adult stages of *Musca domestica* was also inhibited by Chinese star anise (Sripongpun, 2008).

Nowadays, the plant essential oils (EOs) are used as biopesticides - the successful commercial production of plants, they are also considered eco-friendly (Isman, 2020). However, plant essential oils are easily degraded by external factors such as air, light, humidity, and high temperature. For this reason, nEOs were developed to overcome the disadvantages of EOs with nanoemulsion technology. For this reason, turmeric and star anise nEOs were developed in this study. The results also were in accordance with the previous research. Mustafa and Hussein (2020) showed that pesticide nanoemulsions had great potential to develop lipophilic active - loaded products and the advance and chance in growing nanoemulsions as carries or nano delivery system for plant conservation. Tang *et al.* (2013) showed that the efficiency of nanoemulsion could be reached up to 60% towards wild cabbage. Bian (2013) showed that the product had improved insecticidal activity of nanoemulsion towards diamondback moth. Shen *et al.* (2012) showed that the average size of 11 nm of nanoemulsion had a good killing insect effect with low environmental pollution. Also, Chen *et al.*, 2012 demonstrated that the 10-100 nm of particle size of nano-emulsion had a good novel agrochemical application system.

Therefore, turmeric and star anise nEOs can increase the efficiency of cutworm control, and can be used as biopesticides to reduce or eliminate the use of chemical pesticides which cause contamination and residues in plants and environment.

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