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Insecticidal effect of plant essential oil nanoemulsions on controlling *Spodoptera exigua*

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Abstract The results showed that *Curcuma longa* and *Illicium verum* nEOs performed extremely high mortality and antifeedant activity, as well as affected on growth development of *Spodoptera exigua*. Essential oil nanoemulsions at 0.35 % concentrations caused 100% mortality, 100% antifeedant activity, and 100% growth inhibition. *Curcuma longa* and *Illicium verum* nEOs revealed to be used as botanical insecticide for controlling *Spodoptera exigua*.

Keywords: Nanoemulsion, Insecticide, Mortality, Antifeedant activity, Growth inhibition

Introduction

Spodoptera exigua (beet armyworm) is a major pest of agricultural crops (Mitchell, 1973), especially, in ASEAN countries. The main problem of agricultural production loss is destruction by insect pest, which can result in big production damages (Haff and Slaughter, 2004, Fornal *et al*., 2007). The larvae of *Spodoptera exigua* can spread rapidly and attack vegetables. Agriculturists commonly use chemical insecticide to control the pest for the reason that it's simple and easily operated, and provides fast results (Talekar and Shelton, 1993). However, using chemical insecticide for it takes a long time resulting in a negative impact on the environment because of its toxic residue. Moreover, it can cause pesticide resistance. For these reasons, plant essential oils are alternatives to reduce and eliminate the use of chemical insecticide.

Plant essential oils (PEOs) or herbicides can be used as a biological pest control (Elshafie and Camele *et al*., 2017). PEOs are extracted from parts of plant which contain volatile organic compounds (Chen *et al*., 2022), considered eco-friendly, and have less effect on mammalians and environment (Mossa, 2016), they contain monoterpenoid compound, having insecticidal effect on the target pest (Houghton *et al*., 2006), but PEOs can be quickly degradation by

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uncontrollable factors such as temperature, sunlight and have low water solubility (Elshafie *et al*., 2019). Therefore, plant essential oil nanoemulsions have been developed to overcome the disadvantages of plant essential oils.

Nanoemulsions have small-size droplet, with the diameter less than 100 nm (Gupta *et al*., 2016). Formation of nanoemulsion is also used a technique for capsule formulation and drug delivery (Kumar *et al*., 2016). Essential oil nanoemulsions (nEOs) are made on physical fixable, water spreading, and covering surface area (Joe *et al*., 2012). In nanoemulsion formation process, surfactant and co-surfactant were used to increase the stability. The hydrophilic lipophilic balance (HLB) estimated of surface active agent is less than 10. Co-surfactant is mixed to increase the stability of nanoparticle in environment (Rehman *et al*., 2020). The nEOs are harmless to environment and other organism (Anjali *et al*, 2012) and they were used to control pests (Abbott, 1925; Finney, 1971; Van Asperen, 1962; Habig *et al*., 1974).

The aim was to estimate the effect of plant essential oil nanoemulsions in managing beet armyworm, *Spodoptera exigua*.

Materials and methods

Larvae breeding

Larval of the beet armyworm (*Spodoptera exigua*) was collected from cantonese (*Brassica chinensis*) plot in Thailand. They were bred in insect box at room temperature at Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand.

Plant material screening

Twelve selected plant essential oils (EOs) were screened to evaluate their insecticidal property using leaf dip bioassay. A diameter of 3 cm of cantonese leaves were dipped into 1 % and 10% concentrations EOs (1 minute), and dried at room temperature (15 minutes). After that 10 second-stage larvae of *Spodoptera exigua* were put in the testing box. The mortality then was observed 24 hrs. EOs used in this study were prepared in keeping with principles after of hazard analysis and critical control point (HACCP) and procured from the Thai - China Flavors and Fragrances Industry Co. Ltd., Thailand. The 2 most effective plant essential oils were then selected to develop into nanoemulsions and further bioassay test.

Preparation of essential oil nanoemulsions

To prepare the essential oil nanoemulsions, the selected Eos of *Curcuma longa* and *Illicium verum*, were diluted with water and polysorbate (Tween - 20 or nonyl phenol ethoxylate (NP9) as surfactant. In this study, the *Curcuma longa* essential oil nanoemulsion consisted of a ratio of 1:2 turmeric essential oil and NP9. The star anise essential oil nanoemulsion consisted of a ratio of 1:4 star anise essential oil and Tween - 20. Particles sizes of turmeric and star anise nEOs were measured by size analyst. (NanoPlus Zeta, Otsuka Electronic Co., Ltd., Osaka, Japan).

Mortality test

The cantonese leaves were dipped into *Curcuma longa* and *Illicium verum* nEOs at these concentrations 0.00%, 0.10%, 0.15%, 0.20%, 0.25%, 0.30%, and 0.35% for a minute, then dehydrated at room temperature for fifteen minutes. After that 10 second-stage larvae of *Spodoptera exigua* were put in the tested box. The mortality then was investigated after twenty-four hours. The mortality rate was estimated according to 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 (%). Finally, the result was compared to the control group.

Antifeedant effect test

The prepared cantonese leaves were similar to the mortality test method when they were dipped into *Curcuma longa* and *Illicium verum* nEOs at these concentrations 0.00%, 0.10%, 0.15%, 0.20%, 0.25%, 0.30%, and 0.35% for a minute, and dehydrated at room temperature for fifteen minutes. After that 10 second-stage larvae of *Spodoptera exigua* were put in the testing box. The consumed area was measured to calculate the percent of inhibited eating, described by antifeedant index (AFI) (Escoubas *et al.*, 1992) according to the following formula.

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

where C and T are control and test leaf area eating, respectively.

Growth inhibition test

The prepared cantonese leaves were served for *Spodoptera exigua* by the same way. The developmental period of pupa and adult stage of 10 second-stage larvae was observed and compared to the control group.

Statistical analytical

The data were studied by SAS software (SAS Institute, Cary, North Carolina, USA), using Analyst of Variance (ANOVA) and Duncan's New Multiple Range Test (DMRT)

Results

After testing 12 kinds of essential oils, 100 % motality was found at 1% concentrations of *Curcuma longa* and *Illicium verum* essential oils (Table 1). In order to make the essential oil nanoemulsions, Tween-20 and NP9 surfactant were used in this study. The hydrophilic-lipophilic balance (HLB) of Tween-20 was 16.7 and and NP9 was 12.9. *Curcuma longa* nEO consisted of 1% Curcuma longa essential oil and 2% NP9. Its size and zeta potential were 16.1 nm and - 6.15, respectively. *Illicium verum* nEO consisted of 1% *Illicium verum* essential oil and 4% Tween - 20 (surfactant). Its size and zeta potential were 93.2 nm and 1.27, respectively. The interesting result, when 0.35% concentrations of *Curcuma longa* and *Illicium verum* nEOs demonstrated the insect mortality of 100% (Table 2). At 0.35% concentrations of those 2 nEOs presented the antifeedant activity of 100% (Table 3) and showed the growth inhibition effect with 100% (Table 4 and 5). The growth developmental period from larva to pupa and from pupa to adult stages were 5.90 $\pm$ 0.74 days and 7.90 $\pm$ 0.57 days, respectively appeared at 0.30% concentrations of *Curcuma longa* nEOs (Table 4 and 5). Besides, the growth developmental period of pupa stage was 5.80 $\pm$ 0.79 day and adult stage was 7.70 $\pm$ 0.67 days, happened at 0.20% concentrations of *Illicium verum* nEO (Table 4 and 5). Generally, these results showed a reduced in growth developmental period and compared to the control group. However, according to these results, the effect of *Curcuma longa* nEO was not significantly different from that of *Illicium verum* nEO.

Table 1. Percentage of average mortality effect of essential oils from 12 plants on *Spodoptera exigua* larvae after twenty-four hours using leaf dip bioassay

Essential oils (EOs)	**Average mortality percent (Mean (%) ± SD)**	
	Concentrations (%)	
	1	**10**
Amomum krervanh	13.33 ± 0.58^{Bd}	26.67 ± 0.58^{Ad}
Eucalyptus globulus	10.00 ± 0.00^{Bd}	20.00 ± 0.00^{Ad}
Ocimum tenuiflorum	70.00 ± 0.00^{Bb}	100.00 ± 0.00^{Aa}
Foeniculum vulgare	70.00 ± 0.00^{Bb}	100.00 ± 0.00^{Aa}
Piper betle	20.00 ± 0.00^{Bc}	33.33 ± 0.58^{Ac}
Cinnamomum verum	23.33 ± 0.58^{Ac}	26.67 ±0.58^{Ad}
Cymbopogon citratus	23.67 ± 0.58^{Bc}	56.67 ± 0.58^{Ab}
Illicium verum	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
Syzygium aromaticum	20.00 ± 0.00^{Bc}	33.33 ± 0.58^{Ac}
Piper longum	20.00 ± 0.00^{Bc}	36.67 ± 0.58^{Ac}
Piper nigrum	23.33 ± 0.58^{Bc}	33.33 ± 0.58^{Ac}
Curcuma longa	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
Control	0.00 ± 0.00^{Ae}	0.00 ± 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 *Spodoptera exigua* larvae caused by nanoemulsion essential oils after twenty-four hours using the leaf dip bioassay

nEOs	**Average mortality percent (Mean (%) ± SD)**							**LC50**
	Concentrations (%)							
	0.00	**0. 10**	**0. 15**	**0.20**	**0.25**	**0.30**	**0.35**	
Curcuma longa	0.00 ± 0.00^{Fa}	0.00 ± 0.00^{Fa}	26.67 ± 0.58^{Ea}	56.67 ± 0.58^{Da}	60.00 ± 0.00^{Ca}	80.00 ± 0.00^{Ba}	100.0 ± 0.00^{Aa}	0.214
Illicium verum	0.00 ± 0.00^{Ea}	0.00 ± 0.00^{Ea}	26.67 ±0.58^{Da}	56.67 ±0.58^{Ca}	56.67 ±0.58^{Ca}	76.67 ± 0.58^{Ba}	100.0 ±0.00^{Aa}	0.217

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 antifeedant effect of essential oil nanoemulsions (nEOs) on *Spodoptera exigua* in percent after twenty-four hours using the leaf dip bioassay

nEOs	Average antifeedant effect percent (Mean (%) ± SD)						
	Concentrations (%)						
	0.00	**0. 10**	**0. 15**	**0.20**	**0.25**	**0.30**	**0.35**
Curcuma longa	0.00 ± 0.00^{Fa}	10.30 ± 1.16^{Ea}	24.67 ± 2.46^{Da}	45.00 ± 1.81^{Ca}	40.00 ± 0.00^{Ca}	80.00 ± 0.00^{Ba}	100.0 ± 0.00^{Aa}
Illicium verum	0.00 ± 0.00^{Fa}	11.00 ± 1.01^{Ea}	30.00 ± 0.00^{Da}	46.00 ± 1.78^{Ca}	40.00 ± 0.00^{Ca}	80.00 ± 0.00^{Ba}	100.0 ± 0.00^{Aa}

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 inhibition percentage and developmental period of pupa of *Spodoptera exigua* affected by essential oil nanoemulsions (nEOs) after twenty-four hours using the leaf dip bioassay

nEOs	Average inhibiting to growth percent						
	Concentrations (%)						
	Average growth inhibition to pupae (Mean (%) ± SD)						
	0.00	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**
Curcuma longa	0.00 ± 0.00^{Fa}	0.00 ± 0.00^{Fa}	26.67 ± 0.58^{Ea}	43.33 ± 0.58^{Da}	53.33 ± 0.58^{Ca}	70.00 ± 0.00^{Ba}	100.00 ± 0.00^{Aa}
Illicium verum	0.00 ± 0.00^{Fa}	0.00 ± 0.00^{Fa}	$26.67 +- 0.58^{Ea}$	46.67 ± 0.58^{Da}	60.00 ± 0.00^{Ca}	70.00 ± 0.00^{Ba}	100.00 ± 0.00^{Aa}

nEOs	Concentrations (%)						
	Pupa developmental period (days) (Mean ± SD)						
	0.00	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**
Curcuma longa	7.00 ± 0.00^{Aa}	6.80 ± 0.42^{Ba}	6.70 ± 0.52^{Ba}	6.50 ± 0.53^{Ba}	6.20 ± 0.42^{Ba}	5.90 ± 0.74^{Ca}	0.00 ± 0.00^{Da}
Illicium verum	7.00 ± 0.00^{Aa}	6.70 ± 0.48^{Ba}	$6.70 +- 0.52^{Ba}$	6.30 ± 0.48^{Ba}	6.10 ± 0.32^{Ca}	5.80 ± 0.79^{Da}	0.00 ± 0.00^{Ea}

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 inhibition percentage and developmental period of adult of *Spodoptera exigua* affected by essential oil nanoemulsions **(**nEOs**)** after twenty-four hours using the leaf dip bioassay

nEOs	**Average inhibiting to growth percent**						
	Concentrations (%)						
	Average growth inhibition to adult (Mean (%) ± SD)						
	0.00	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**
Curcuma longa	0.00 ± 0.00^{Fa}	0.00 ± 0.00^{Fa}	30.00 ± 0.00^{Ea}	50.00 ± 0.00^{Da}	70.00 ± 0.00^{Ca}	83.33 ± 0.58^{Ba}	100.00 ± 0.00^{Aa}
Illicium verum	0.00 ± 0.00^{Fa}	0.00 ± 0.00^{Fa}	30.00+- 0.00^{Ea}	53.33 ± 0.58^{Da}	70.00 ± 0.00^{Ca}	86.67 ± 0.58^{Ba}	100.00 ± 0.00^{Aa}
nEOs	**Concentrations (%)**						
	Adult developmental period (days) (Mean ± SD)						
	0.00	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**
Curcuma longa	9.20 ± 0.42^{Aa}	9.11 ± 0.78^{Aa}	9.00 ± 0.82^{Aa}	8.70 ± 0.48^{Ba}	8.30 ± 0.48^{Ba}	7.90 ± 0.57^{Ca}	0.00 ± 0.00^{Da}
Illicium verum	9.20 ± 0.42^{Aa}	9.10 ± 0.32^{Aa}	8.90+- 0.74^{Aa}	8.60 ± 0.52^{Ba}	8.20 ± 0.42^{Ba}	7.70 ± 0.67^{Ca}	0.00 ± 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.

Discussion

These results were in corresponds to many reports as followed. Compounds of black mustard and turmeric had the most effective on mortality of beet armyworm (Tavares de *et al*., 2019). The turmeric extract presented the most effect on the mortality of adult stage of red flour beetle (Abida *et al*., 2010). Also, this turmeric extract performed inhibited effect of growth control desert locust and red cotton bug (Chowdhury *et al*., 2000). Other plant extracts as of, anise and fennel showed strong antifeedant activity, and growth inhibition effect on gypsy moth (Kosti ́c *et al*., 2021), the *Cuminun cyminum* and *Pimpinella anism* caused toxicity effects on many insect pests (Benelli *et al*., 2018). Insecticidal property of many plant extracts against *Spodoptera* sp. were also reported as, clove, eucalyptus, fennel, lettuce, and mint oils showed 52.58% antifeedant effect on third-stage instar larva of *Spodoptera littoralis* (Sabbour and Shadia, 2002). Neem, citronella, castor, and clove EOs had toxic effects on 2nd stage instar larvae of *Spodoptera littoralis* (Dono *et al*., 2020). Biopesticide from essential oils (EOs) are environmentally friendly (Isman, 2020), but they are quickly degradation by uncontrollable factors such as temperature, sunlight, and moist. As a result, EOs were expanded into essential oil nanoemulsions (nEOs) for several advantages. The nEOs have small particles, but high stability due to oil encapsulation, and high surface area of emulsions, compared to the essential oils (Jonassen *et al*., 2012). The nEOs are also considered environmentally friendly (Chinnamuthu and Boopathi, 2009). The pesticide nanoemulsion formulation is the new formulation technology. The nEOs can be formed without using a homogenizer, the cost of which is high, by the method called spontaneous emulsification by stirring oils, using surfactants and water at a proper ratio for maximum efficiency, resulting in lower cost (Ariyaprakai, 2017). Jiang (2016) showed that the products of nanoemulsion have been used as insecticide for field application.

Therefore, using *Curcuma longa* and *Illicium verum* essential oil nanoemulsions can increase the effectiveness in controlling *Spodoptera exigua*. In the future, it will possible be used as an alternative method for *Spodoptera exigua* control at the farm and plot to reduce and eliminate the use of chemical insecticides.

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