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Toxicity of Nano emulsions of Clove (*Syzigium aromaticum* L.) and Nutmeg (*Myristica fragrans* Houtt.) Essential Oil Against *Spodoptera frugiperda* J.E. Smith

Eka Wulandari[1] , Ni Siluh Putu Nuryanti[2*], Desi Maulida[3]
[1,2,3]*Master of Food Security Study Program, Politeknik Negeri Lampung Bandar Lampung, 35144, Indonesia*
*Corresponding author: niluh@polinela.ac.id

Abstract

Spodoptera frugiperda is a significant maize pest that significantly reduces crop yields. Conventional control typically involves chemical insecticides, which can lead to environmental harm and food safety concerns. Therefore, eco-friendly alternatives such as botanical insecticides are needed. This study evaluated the toxicity of nanoemulsions of clove (*Syzigium aromaticum*) and nutmeg (Myristica fragrans) essential oils against third-instar larvae of *S. frugiperda*. The research methods included rearing *S. frugiperda*, extracting essential oils, formulating nanoemulsions, and conducting toxicity tests. Analysis using Polo-PC showed that both LC_{50} and LC_{95} values decreased over the observation period from 24 to 96 hours after treatment (HAT). For clove essential oil nano emulsion, LC_{50} and LC_{95} values were 0.52% and 7.16% (24 HAT); 0.43% and 5.03% (48 HAT); 0.36% and 4.14% (72 HAT); and 0.35% and 4.02% (96 HAT). For nutmeg essential oil nano emulsion, LC_{50} and LC_{95} values were 1.43% and 10.20% (24 HAT); 1.10% and 10.51% (48 HAT); 0.90% and 11.23% (72 HAT); and 0.60% and 6.06% (96 HAT). Based on the LC_{50} and LC_{95} values, the study concludes that clove and nutmeg essential oil nanoemulsions have potential as botanical insecticides against *S. frugiperda* larvae and offer a safer alternative for the environment and human health.
Keywords: clove, nano emulsion, nutmeg, *Spodoptera frugiperda*, toxicity.

Introduction

The Fall Armyworm (*Spodoptera frugiperda*) is an invasive pest believed to originate from the tropical climate of South America (Nonci et al., 2019). In early 2019, this pest was discovered infesting corn plants in the Sumatra region (Subiono, 2019). It attacks the plant's growing point, potentially leading to the failure to form plant shoots or young leaves. The larvae of *S. frugiperda* have a high feeding capacity. They bore into plant tissues and feed actively inside, making early detection difficult when population levels remain low. Adult moths are strong fliers and capable of traveling long distances. The potential yield loss in corn caused by *S. frugiperda* infestation is estimated to reach 8.3 to 20.6 million tons per year if no control efforts are taken (FAO & CABI, 2019).

Farmers commonly control *S. frugiperda* using synthetic insecticides (Baudron et al., 2019). However, excessive and improper use of synthetic insecticides can lead to environmental pollution (soil and water contamination, harm to wildlife and other organisms) and pose health risks to farmers, such as dizziness, nausea, and other symptoms (Dhaifulloh et al., 2024). Therefore, there is a need for safer and more environmentally friendly insecticide alternatives, such as plant-based insecticides. These botanical insecticides, derived from plant extracts, contain secondary metabolite compounds that act as repellents, deterrents, toxic agents, or growth inhibitors (Arsy et al., 2023).

Examples of plants that contain such secondary metabolites are clove and nutmeg. Clove essential oil contains two main components: eugenol (74.28%) and trans-caryophyllene (20.49%). The main components of nutmeg essential oil include methyl eugenol, myristicin, safrole, and elemicin, which have been shown to have toxic effects on insects, functioning as both contact and fumigant insecticides (Cossetin et al., 2021). Nutmeg essential oil also has potential as an antifeedant compound that can suppress insect appetite and hinder their growth (Erza et al., 2022).

According to research by Purwanto et al. (2024), nutmeg seed essential oil has higher toxicity against *S. zeamais* than citronella and temulawak essential oils. One drawback of using essential oil-based insecticides is their volatile nature (Aryani, 2020). To improve their stability, the active compounds in essential oils need to be formulated into more stable forms, such as through nanotechnology (Nuryanti et al., 2018).

Nanoemulsions are stable systems with particle sizes ranging from 1 to 100 nm (Pratiwi et al., 2018). Nanoemulsions can enhance the solubility of natural substances and improve absorption variability, permeability, and bioavailability (Chime et al., 2014). The success of nanoemulsion production greatly depends on the components of the emulsion formula, such as the active ingredients and the emulsifier concentration used (Balaj & Zarzar, 2020).

In recent years, research on botanical insecticides has grown considerably. However, studies that specifically examine the use of clove and nutmeg essential oil nanoemulsions are still limited. Therefore, research on the toxicity of clove and nutmeg essential oil nanoemulsions against *S. frugiperda* is needed.

Research Method

The study was conducted at the Plant Laboratory 1, Politeknik Negeri Lampung, from June to December 2024. The equipment utilized included a blender, measuring cylinder, oviposition cages (dimensions: 108 cm × 65 cm × 100 cm), rotary evaporator, distillation apparatus, 5-liter amber glass bottles, sieves, filter papers, measuring cylinders, hand sprayer (for contact bioassay), tweezers, small brushes, plastic cups (5 cm height, 9 cm diameter), beakers, glass funnels, glass spatulas, scissors, cutter knives, drafting paper, 100 mL plastic bottles, transfer pipettes, and square trays. The materials used consisted of nutmeg seeds sourced from Purwakarta (West Java), clove flowers obtained from Subang (West Java), Tween 80 as an emulsifier, third-instar larvae of *Spodoptera frugiperda*, baby corn, corn leaves, planting media in polybags for oviposition, methanol, distilled water, and honey.

Insect Preparation

The rearing of *S. frugiperda* larvae followed the protocol by the Directorate General of Food Crops (2023), where eggs were deposited in clusters on young corn leaves, hatched in a single container, and subsequently fed with baby corn and corn leaves. Larvae used for treatments were at the third instar stage.

Distillation of Clove and Nutmeg Essential Oils

Clove flowers and nutmeg seeds were ground using a blender (type FF023) into fine powder and subsequently air-dried for 2–3 days. After drying, steam distillation was performed following the method described by Puspa et al. (2017). Distillation was carried out at approximately 130 °C for 5–6 hours until no further oil droplets were observed at the condenser outlet. The distillate was then allowed to stand to enable complete separation of the essential oil and water layers.

Nanoemulsions Formulation

Nanoemulsions were prepared using a low-energy emulsification method via phase inversion, adapted from Ostertag et al. (2012) with modifications involving gradually adding the aqueous phase into the organic phase. The formulation consisted of clove and nutmeg essential oils blended with Tween 80 in a 1:1 ratio. Two millilitres of each essential oil were mixed with 2 mL of Tween 80 in a measuring cylinder and stirred at 500 rpm using a magnetic stirrer. Distilled water (96 mL) was added dropwise (at a rate of 4 mL/min) while continuously stirring. After complete addition, the emulsion was homogenized at 250 rpm for 10 minutes.

Toxicity Assay of Essential Oil Nanoemulsions Against *S. frugiperda*

A Completely Randomized Design (CRD) was employed. The first factor was the type of nanoemulsions: N1 = Clove essential oil nanoemulsions, N2 = Nutmeg essential oil nanoemulsions. The second factor was the concentration of the nanoemulsion solution: CK0 = Control, K1 = 0.125%, K2 = 0.25%, K3 = 0.5%, K4 = 1%, K5 = 2% (according to Nuryanti et al., 2021). Each treatment combination was replicated three times, resulting in 36 experimental units. Ten third-instar *S. frugiperda* larvae were placed in each container and sprayed with the corresponding nanoemulsions treatment. Mortality data were recorded 24-, 48-, 72-, and 96 HAT. Data were analyzed using probit analysis with the Polo-PC software to estimate LC50 and LC95 values.

Results and Discussion

Toxicity of Clove Essential Oil Nanoemulsions

Polo-PC analysis indicated that LC50 and LC95 values decreased progressively from 24 to 96 HAT (Table 1). At 24 HAT, LC50 and LC95 values were recorded at 0.52% and 7.16%, respectively. On the second day, these values declined to 0.43% and 5.03%. By the third day, further reductions were observed at 0.36% and 4.14%. On the final observation day, LC50 and LC95 values reached 0.35% and 4.02%, respectively. This consistent downward trend in LC values indicated increased larval mortality over time, demonstrating the toxic effect of the clove essential oil nanoemulsions against *S. frugiperda* larvae.

Table 1. Estimation of Probit Analysis Parameters for Clove Essential Oil against *Spodoptera frugiperda*

Essential Oils	HAT	a^a±GB	b^b±GB^c	LC^d_{50} (SK^e95%) (%)	LC^d_{95} (SK^e95%) (%)
Cloves	24	1,44 ± 0,27	0,28 ± 0,75	0,52 (0,36-0,80)	7,16 (3,13-40,80)
	48	1,53 ± 0,27	0,30 ± 0,76	0,43 (0,30-0,61)	5,03 (2,45-21,28)
	72	1,56 ± 0,27	0,32 ± 0,76	0,36 (0,26-0,51)	4,14 (2,10-15,60)
	96	1,56 ± 0,27	0,32 ± 0,76	0,35 (0,25-0,49)	4,02 (2,06-14,50)

Notes : a^a = probit regression intercept, b^b = probit regression slope, GB^c = standard error, LC^d = lethal concentration, SK^e = confidence interval, HAT = Hours After Treatment

LC_{50} refers to the concentration or dose that causes 50% mortality of test insects at a specific observation time (Hasyim et al., 2016). The smaller the LC value, the more toxic the botanical insecticide is considered to be (Hasyim et al., 2019). Research conducted by Sutikno and Anggraini (2023) reported that clover leaf extracts using organic solvents applied to *Spodoptera litura* had LC_{50} values ranging from 0.06% to 0.32%. Another study also demonstrated the efficacy of clove essential oil against *Ephestia cautella*, achieving nearly 100% toxicity (Shaimaa & Mahdi, 2024). The secondary metabolites contained in clove essential oil are primarily eugenol (74.28%) and trans-caryophyllene (20.49%) (Sitohang et al., 2018). The mechanism of action of eugenol involves disrupting the respiratory and nervous systems of insects, leading to paralysis and death. Additionally, eugenol is an antifeedant that prevents insects from consuming the provided food (Mika Mega Astuthi et al., 2013).

The chemical compounds interfere with the pest's digestive system and disrupt their metabolic processes, ultimately causing paralysis, loss of appetite, and death. Moreover, the regression parameters "a" and "b" indicated a stable dose-mortality relationship with minimal changes over time. The relatively small "b" value suggests that mortality was not drastic at low concentrations but increased significantly at higher concentrations. This finding demonstrates that the effectiveness of clove essential oil nanoemulsions increases over time, exerting a greater impact on the mortality of *S. frugiperda* during prolonged exposure (Sutikno & Anggraini, 2023).

Toxicity of Nutmeg Essential Oil Nanoemulsions

Applying nutmeg essential oil nanoemulsions at 24-, 48-, 72-, and 96-hours after-treatment (HAT) exhibited toxic effects on third-instar larvae of *Spodoptera frugiperda*. Across all observation times, the LC_{50} and LC_{95} values decreased (Table 2). At 24 HAT, the LC_{50} value was 1.43% with a confidence interval of 1.07–2.07%, while the LC_{95} value was 10.20% with an interval of 5.54–31.05%. On the second day (48 HAT), the LC_{50} and LC_{95} values decreased to 1.10% and 10.51%, respectively. On the third day (72 HAT), the LC_{50} further decreased to 0.90%, with the LC_{95} recorded at 11.23%. By 96 HAT, the LC values showed a more substantial reduction, with the LC_{50} value reaching 0.60% and the LC_{95} value at 6.66%. Over time, this trend of decreasing LC values indicates an increasing mortality rate among *S. frugiperda* larvae following prolonged exposure to the nutmeg essential oil nanoemulsions.

Table 2. Estimate of Probit Analysis Parameters for Nutmeg Essential Oil against *S. frugiperda*

Essential Oils	HAT	a^a±GB	b^b±GB^c	LC^d_{50} (SK^e95%) (%)	LC^d_{95} (SK^e95%) (%)
Nutmeg	24	1,93 ± 0,30	0,12 ± 0,93	1,43 (1,07-2,07)	10,20 (5,54-31,05)
	48	1,68 ± 0,27	0,64 ± 0,75	1,10 (0,81-1,60)	10,51 (5,35-36,88)
	72	1,50 ± 0,26	0,83 ± 0,68	0,90 (0,64-1,33)	11,23 (5,33-48,04)
	96	1,58 ± 0,26	0,12 ± 0,69	0,60 (0,42-0,84)	6,66 (4,54-21,68)

Notes: a^a = probit regression intercept, b^b = probit regression slope, GB^c = standard error, LC^d = lethal concentration, SK^e = confidence interval, HAT = Hours After Treatment

Observations made at 24-, 48-, 72-, and 96 HAT revealed that nutmeg essential oil applied at various concentrations exhibited toxic effects on third-instar *Spodoptera frugiperda* larvae. This finding aligns with the study by Nuryanti et al. (2023), which reported that essential oil from *Myristica fragrans* applied to *Sitophilus zeamais* exhibited the highest toxicity in contact treatments, with an LC_{50} value of 0.53% and an LC_{95} value of 4.38%. Research by Faheem & Abduraheem (2019) demonstrated that the growth of *Anthrenus verbasci* larvae was significantly affected by applying nutmeg oil through direct contact and digestive toxicity within 24 hours post-application. Furthermore, another study concluded that nutmeg oil was toxic to adult *Chrysomya albiceps* (topical toxicity and fumigant toxicity) and also exhibited insecticidal effects on both *Musca domestica* larvae and adults (Cossetin et al., 2021).

This study carefully recorded the average and standard deviation values for parameters "a" and "b," in addition to the lethal concentrations needed to kill 50% and 95% of the *S. frugiperda* larvae population. A critical interpretation of the "a" value at 24, 48, 72, and 96 hours revealed fluctuating trends, initially decreasing from 1.93 ± 0.30 to 1.50 ± 0.26, then slightly increasing to 1.58 ± 0.26. This fluctuation indicates a complex interaction between active compounds and the physiological responses of insects over time. This non-linear development may indicate the initial high susceptibility of insects to the nutmeg extract, followed by gradual development of tolerance or detoxification mechanisms, or possibly due to the degradation of active compounds.

The "b" values also exhibited variability, starting at 0.12 ± 0.93, increasing to 0.83 ± 0.68, and then decreasing to 0.12 ± 0.69, highlighting the dynamic nature of the insecticidal effects, which may be related to the diverse properties of the compounds in the nutmeg extract or changes in the metabolic rate of the insects (Ntalli & Caboni, 2012).

Nutmeg essential oil contains Beta-pinene (26%), Alpha-pinene (10.5%), and Sabinene (9.1%) as its major compounds (Cossetin et al., 2021), along with secondary metabolites such as flavonoids and tannins (Erza et al., 2022). GC-MS analysis of nutmeg essential oil detailed the presence of 14 monoterpene compounds, including Alpha Phellandrene, Beta-Ocimene, Sabinene, and 2-beta.-pinene, Myrcene, Beta-Phellandrene, Trans-Beta Ocimene, Alpha Terpinene, p-cymene, Limonene, Gamma-Terpinene, Alpha-Terpinolene, Terpinene-4-ol, and Myristicin (Pareta, 2022). Among these compounds, Myristicin is believed to be the main component responsible for the toxic effects on insects (Hallström & Thuvander, 1997).

Conclusion

Nanoemulsions of clove (*Syzigium aromaticum*) and nutmeg *(Myristica fragrans*) essential oils exhibited toxicity against *Spodoptera frugiperda* larvae. The LC_{50} value for clove essential oil at 96 HAT was 0.35%, while the LC_{50} for nutmeg essential oil was 0.60%. With a lower LC_{50} value, clove essential oil proved more effective than nutmeg oil, making it a preferred choice for bioinsecticide formulations. Natural compounds like eugenol and myristicin provide an environmentally friendly alternative for controlling *S. frugiperda*. However, further development of formulations and field applications is necessary to improve effectiveness, stability, and product durability under various environmental conditions.

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