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RESEARCH PAPER

Activity of nanoemulsion formulated from *Syzygium aromaticum* and *Myristica fragrans* essential oils against *Spodoptera frugiperda*

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ABSTRACT

Spodoptera frugiperda is a major pest of maize that can causes severe yield losses, while intensive use of synthetic insecticides raises concerns about environmental contamination and food safety. This study evaluated the larvicidal toxicity, synergistic interaction, feeding inhibition, and egg-hatching inhibition of nanoemulsions formulated with *Syzygium aromaticum* and *Myristica fragrans* essential oils at ratios of 1:1, 2:1, and 1:2 (v/v). The nanoemulsions were prepared using a low-energy phase-inversion method with Tween 80 as the emulsifier. Larvicidal activity against third-instar *S. frugiperda* was evaluated using a contact bioassay at concentrations of 0, 0.125, 0.25, 0.50, 1.00, and 2.00% (v/v), and mortality was recorded for 96 hours after treatment (HAT). Larvicidal potency was estimated by probit analysis, while interactions between the essential oils were evaluated using the combination index (CI). Feeding inhibition and egg-hatching inhibition were also assessed at a total essential oil concentration of 1.00%. The 2:1 *S. aromaticum: M. fragrans* nanoemulsion exhibited the greatest larvicidal potency, with an LC_{50} of 0.10% and LC_{95} of 1.42% at 96 HAT, and showed strong synergism (CI = 0.26 at LC_{50}). This formulation had a mean droplet size of 95.5 nm. At 1.00% total essential oil concentration, the 2:1 formulation inhibited egg hatching by 88% and reduced larval feeding, with the feeding inhibition index reaching 61.02%. These findings demonstrate the potential of the 2:1 *S. aromaticum: M. fragrans* nanoemulsion as a botanical insecticide candidate for the management of *S. frugiperda*. Further studies on chemical composition, formulation stability during storage, and efficacy under greenhouse and field conditions are needed to support its development as a botanical insecticide.

Keywords: Botanical insecticides, essential oil, nanoemulsion, phase inversion, *Spodoptera frugiperda*

INTRODUCTION

Nanotechnology has emerged as a promising approach for improving the efficacy and applicability of botanical pesticides in agriculture. Among nanotechnology-based delivery systems, nanoemulsions have received considerable attention because they can improve the dispersion, stability, and bioavailability of hydrophobic bioactive compounds. Nanoemulsions are colloidal dispersions consisting of two immiscible liquid phases, typically oil and water, stabilized by surfactants, with droplet sizes generally ranging from 20 to 200 nm. Compared with conventional emulsions, their small droplet size provides a larger interfacial surface area and may enhance the interaction of active compounds with target organism (de Lima et al., 2021).

Nanoemulsions can be produced using either high-energy or low-energy methods. Low-energy approaches, including Phase Inversion Composition (PIC) and spontaneous emulsification, have attracted increasing interest because they require relatively low energy input and mild processing conditions. These characteristics are particularly advantageous for the formulation of essential oils, whose volatile and thermolabile components may be susceptible to degradation during processing (Sheikh et al., 2021). Low-energy methods can also produce fine droplets with a relatively uniform size distribution, potentially improving the dispersion and biological performance of essential oil-based formulations.

The development of effective botanical insecticides is particularly important for controlling *Spodoptera frugiperda* (J.E. Smith), commonly known as the fall armyworm. This pest has become a major global threat to agricultural production because of its high reproductive capacity, rapid development, broad host range, and substantial feeding damage (Togola et al., 2025). Its management has relied heavily on synthetic insecticides (Baudron et al., 2019). However, intensive and inappropriate pesticide use may result in environmental contamination, adverse effects on non-target organisms, and potential risks to human health

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(Dhaifulloh et al., 2024). Moreover, the repeated use of insecticides with similar modes of action can contribute to the development of insecticide resistance. These concerns highlight the need for alternative pest management strategies based on effective and environmentally compatible bioactive compounds.

Essential oils derived from aromatic plants represent a promising source of botanical insecticides because they contain diverse secondary metabolites, including phenols, terpenoids, and aldehydes, that may act as repellents, antifeedants, contact toxicants, or insect growth regulator (Arsy et al., 2023). Among these plant-derived materials, essential oils from *Syzygium aromaticum* and *Myristica fragrans* have demonstrated considerable insecticidal potential. The essential oil of *S. aromaticum* is characterized primarily by the presence of eugenol and other bioactive compounds, including trans-caryophyllene, which have been associated with insecticidal, antimicrobial, and repellent activities (Wanna et al., 2024). Previous studies have also indicated that nanoformulation may improve the stability and biological activity of *S. aromaticum* essential oil (Iqbal et al., 2025).

Meanwhile, *M. fragrans* essential oil contains several biologically active compounds, including myristicin, safrole, and elemicin, which have been reported to affect insect physiological processes and larval development (Cossetin et al., 2021). In addition to its toxic effects, *M. fragrans* oil may exhibit antifeedant activity and suppress insect growth (Erza et al., 2022). Its insecticidal potential has also been demonstrated against stored-product pests, with higher toxicity against Sitophilus zeamais than citronella and turmeric essential oils in a previous study (Purwanto et al., 2024). Given their different chemical compositions and potential modes of action, combining *S. aromaticum* and *M. fragrans* essential oils may provide an opportunity to enhance insecticidal efficacy through additive or synergistic interactions.

Despite their promising biological activities, the practical application of essential oils is often limited by their high volatility, poor water solubility, and susceptibility to environmental degradation, which may reduce their persistence and effectiveness under field conditions (Wang et al., 2021). Nanotechnology-based formulations offer a potential strategy to overcome these limitations by improving the dispersion and stability of hydrophobic essential oil components in aqueous systems (Chime et al., 2014). Nanoencapsulation or nanoemulsification may also protect sensitive compounds from degradation and enhance their contact with target organisms, thereby improving biological activity (Nuryanti et al., 2023).

Several studies have demonstrated the potential of essential oil nanoemulsions for controlling *S. frugiperda*. For example, a nanoemulsion formulated from *Cedrela odorata* essential oil showed strong larvicidal activity against this pest (Lemus de la Cruz et al., 2022). Similarly nanoemulsions of lemongrass and *Piper aduncum* essential oils prepared using spontaneous emulsification were effective against *S. frugiperda* larvae, with an LC_{50} value of 0.53% (Lina et al., 2023). However, previous studies have predominantly focused on nanoemulsions containing a single essential oil. To the best of our knowledge, the insecticidal potential of nanoemulsions combining *S. aromaticum* and *M. fragrans* essential oils against *S. frugiperda* has not yet been investigated. In particular, information regarding the influence of different oil ratios on toxicity, feeding inhibition, egg hatching, and potential synergistic interactions remains limited.

Therefore, the present study aimed to develop nanoemulsions containing combined *S. aromaticum* and *M. fragrans* essential oils and evaluate their activity against *S. frugiperda*. Specifically, this study investigated the physicochemical characteristics of nanoemulsions formulated with different essential oil ratios and evaluated their insecticidal activity, potential synergistic interactions, feeding inhibition, and effects on egg hatching. The nanoemulsions were prepared using a low-energy method as a strategy to develop a stable and potentially effective essential oil-based formulation for the management of *S. frugiperda*.

MATERIALS AND METHODS

Research Site. This study was conducted at Plant Production Laboratory I, Politeknik Negeri Lampung, Indonesia, where the nanoemulsions were formulated and their biological activities against *S. frugiperda* were evaluated. The particle size and polydispersity index (PDI) of the nanoemulsions were measured at the Integrated Laboratory and Research Center, University of Indonesia (ILRC UI), Indonesia.

Mass Rearing and Maintenance of *S. frugiperda*. Larvae of *S. frugiperda* were collected from the practice field of Politeknik Negeri Lampung and subsequently reared at the Plant Production Laboratory I. The rearing procedure followed the general maintenance guidelines for *S. frugiperda* described by FAO & CABI (2019) and Herlinda et al. (2022).

When the larvae reached the third instar, they were transferred to individual individually into

thin-walled plastic containers (400 mL capacity, approximately 10 cm in height and 9 cm in diameter) containing young corn leaves as food. Individual rearing was applied to prevent cannibalism, and the larvae were maintained until pupations. The pupae were then transferred individually into small glass Petri dishes (approximately 6 cm in diameter and 1.5 cm in height) containing sterile sawdust as a pupation substrate.

The pupae were maintained in gauze cages measuring 1 × 1.5 m until adult emergence. Corn plants grown in polybags were provided inside the cages as oviposition substrates. Cotton soaked in a 10% honey solution was suspended inside each cage as a food source for adult moths. Egg masses deposited on the corn leaves were collected and transferred to plastic jars until hatching. The resulting larvae were maintained under the same rearing conditions and used for subsequent experiments.

Third-instar larvae were used for the toxicity assay because they were sufficiently developed for individual handling and experimental application.

Essential of Essential Oils from *S. aromaticum* and *M. fragrans*. The plant materials used in this study consisted of flower buds of *S. aromaticum* (Myrtaceae) obtained from Subang Regency and mace of *M. fragrans* (Myristicaceae) obtained Bogor Regency, West Java Province, Indonesia. The plant materials were air-dried for 7–14 days, chopped into small pieces, and ground using an FF023-type blender to increase the surface area before extraction.

Essential oils were extracted separately from each plant material using steam distillation according to the method described by Puspa et al. (2017). Water was heated to generate steam, which passed through the plant material contained in the distillation vessel. The steam carrying the volatile compounds was subsequently condensed, and the essential oil was separated from the aqueous phase using a separating funnel. The obtained essential oils were stored in tightly sealed bottles at 4 ºC until further use.

Nanoemulsion Formulation. Nanoemulsions were prepared using a low-energy emulsification method by gradually adding the aqueous phase to the oil phase, following the procedure described by Nuryanti et al. (2023). Each formulation was prepared separately.

The essential oil phase and Tween 80 were mixed at the predetermined volume ratios shown in Table 1, The mixture was homogenized using a magnetic stirrer at 750 rpm while maintaining the temperature below 27 ºC. Distilled water was subsequently added dropwise at a rate of approximately 4 mL/min until the final volume reached 100 mL. The resulting mixture was further homogenized using a magnetic stirrer at 750 rpm for 30 min.

For biological assays, the required nanoemulsion concentration was prepared by diluting the stock nanoemulsion with distilled water. Both single essential oil and mixed essential oil nanoemulsions were prepared at a maximum test concentration of 2% (v/v). Tween 80 was incorporated into each formulation as the emulsifying agent.

Nanoemulsion Activity Analysis.

***Toxicity Assay*.** This toxicity assay was conducted to determine the lethal concentration (LC) values of the nanoemulsion formulations. The procedure was adapted from Nuryanti et al. (2022). Nanoemulsions containing *S. aromaticum* and *M. fragrans* essential oils at three volume ratios (1:1, 2:1, and 1:2) were evaluated (Table 1). Each formulation was tested at six concentrations: 0, 0.125, 0.25, 0.5, 1, and 2%.

Each treatment combination, consisting of essential oil composition and nanoemulsion concentration, was replicated three times. A separate control treatment was included for each formulation to ensure valid comparisons within the experimental design.

The toxicity assay was performed using a contact application method. Ten third-instar larvae of *S. frugiperda* were placed in each plastic container and exposed to the corresponding nanoemulsion formulation using a handheld manual sprayer. The formulation was

Table 1. Composition of the essential oil nanoemulsion formulations tested against *Spodoptera frugiperda*

Formulation	Formulation composition (mL)			Total volume (mL)
	Essential oil composotion (mL)	Tween 80 (mL)	Distilled water (mL)	
Mixed 1:1	*S. aromaticum* (1.00) + *M. fragrans* (1.00)	2.00	96.00	100.00
Mixed 2:1	*S. aromaticum* (1.33) + *M. fragrans* (0.67)	2.00	96.00	100.00
Mixed 1:2	*S. aromaticum* (0.67) + *M. fragrans* (1.33)	2.00	96.00	100.00

sprayed from a distance of approximately 10 cm until the larval body surfaces and corn leaves were evenly moistened without excessive runoff.

Larval mortality was recorded at 24, 48, 72, and 96 hours after treatment (HAT). Mortality data recorded at 96 HAT were subsequently subjected to probit analysis to estimate the LC values of each formulation.

Compatibility and Synergistic Interaction of Essential Oil Mixtures. The interaction between *S. aromaticum* and *M. fragrans* essential oils was evaluated using the combination index (CI) based on the LC values obtained from the toxicity assay. The CI was calculated according to the method proposed by Chou & Talalay (1984):

$$CI = \frac{LC_x^{1cm}}{LC_x^{1}} + \frac{LC_x^{2cm}}{LC_x^{2}} + \left(\frac{LC_x^{1cm}}{LC_x^{1}} + \frac{LC_x^{2cm}}{LC_x^{2}} \right)$$

LC_x^{1} and LC_x^{2} = The LC values of the individual (single) extracts;
$LC_x^{1(cm)}$ and $LC_x^{2(cm)}$ = The LC values of the mixture, multiplied by the proportion of each extract in the mixture that causes mortality (15–95%).

The interaction between the two essential oils was classified according to Kosman & Cohen (1996) as follows:
CI < 0.5 : Strong synergistic effects;
0.5 ≤ CI ≤ 0.77 : Weak synergistic effects;
0.77 < CI ≤ 1.43 : Additive effect;
CI > 1.43 : Antagonistic effect.

Inhibition of Egg Hatching. Egg masses of *S. frugiperda* obtained from laboratory-reared adults were used to evaluate the effect of the nanoemulsion formulations on egg hatching. Each egg mass was placed in a plastic jar (7 cm in diameter and 20 cm in length).

The egg masses were treated with nanoemulsions containing *S. aromaticum* essential oil at 1.00%, *M. fragrans* essential oil at 1.50%, or a 2:1 mixture of *S. aromaticum* and *M. fragrans* essential oils at a total concentration of 1.00%. The treatments were applied using a handheld manual sprayer to ensure that the egg masses were uniformly covered with the test solution.

The number of eggs that successfully hatched was recorded for each treatment. The percentage inhibition of egg hatching was calculated according to Vijitkul et al. (2025):

$$EH\ (\%) = \frac{hk - hp}{hk} \times 100\%$$

EH = Egg hatching inhibition (%);
h_k = Percentage of eggs hatched in the control group;
h_p = Percentage of eggs hatched in the treatment group.

The magnitude of the ovicidal effect was categorized according to Arivoli & Tennyson (2013) as follows:
– : No effect;
+ : < 25% low-grade egg hatching;
++ : 25–50% moderate egg hatching;
+++ : 50–75% high-grade egg hatching;
++++ : > 75% very high-grade egg hatching.

Feeding Inhibition Assay. The antifeedant activity of the nanoemulsion formulations was evaluated using corn leaves as the food substrate. Five corn leaf sections measuring 3 × 3 cm were placed inside a plastic container (7 cm in diameter and 20 cm in length).

The leaf sections were treated with nanoemulsions containing *S. aromaticum* essential oil at 1.00%. *M. fragrans* essential oil at 1.50%, or a 2:1 mixture of *S. aromaticum* and *M. fragrans* essential oils at a total concentration of 1.00%. The formulations were applied using a handheld manual sprayer until the leaf surfaces were uniformly coated. The treated leaves were allowed to air-dry before the insects were introduced.

Ten fifth-instar larvae of *S. frugiperda* were subsequently introduced into each container. Each treatment was replicated three times. The leaf area consumed by the larvae was used as the primary response variable.

Leaf consumption was determined by tracing the remaining leaf area onto millimeter graph paper. The consumed leaf area was calculated by subtracting the remaining leaf area from the original leaf area. The Feeding Inhibition Index (FEI) was calculated according to Hariri (2012):

$$FEI = \left(\frac{(L_c - L_t)}{(L_c - L_t)} \right) \times 100\%$$

FEI = Feeding Inhibition (%);
L_c = Leaf area consumed in the control group;
L_t = Leaf area consumed in the treatment group.

Emulsion Droplet Size and Polydispersity Index (PDI). The mean droplet size and polydispersity index

(PDI) of the nanoemulsion formulations containing *S. aromaticum* and *M. fragrans* essential oils were determined using a Horiba SZ-100 Particle Size Analyzer (PSA) based on the dynamic light scattering (DLS) method (Noor et al., 2015; Pratiwi et al., 2018).

The measurements were performed according to the manufacturer's operating procedure. Mean droplet size was expressed in nanometers (nm), whereas the PDI was used to evaluate the homogeneity of droplet size distribution within each nanoemulsion formulation.

RESULTS AND DISCUSSION

Toxicity of *S. aromaticum* and *M. fragrans* Essential Oil Nanoemulsions Against *S. frugiperda* Larvae. Based on the probit analysis conducted at 96 hours after treatment (Table 2), nanoemulsions containing different ratios of *S. aromaticum* and *M. fragrans* essential oils exhibited different levels of toxicity against *S. frugiperda* larvae. The variation in LC values among the formulations indicates that the relative proportion of the two essential oils influenced their larvicidal potency.

The 1:1 formulation had an LC_{50} of 0.28% and an LC_{95} of 7.74%, whereas the 2:1 formulation showed the lowest LC_{50} and LC_{95} values, at 0.10% and 1.42%, respectively. The 1:2 formulation showed intermediate toxicity, with LC_{50} and LC_{95} values of 0.16% and 2.27%, respectively. Thus, among the tested formulations, the 2:1 ratio exhibited the greatest larvicidal potency based on its lower LC_{50} and LC_{95} values. However, differences in potency should be interpreted based on the confidence intervals and appropriate statistical comparisons rather than LC point estimates alone.

The relatively high toxicity of the 2:1 formulation may be associated with the greater proportion of *S. aromaticum* essential oil in the mixture. *S. aromaticum* essential oil is commonly rich in eugenol, which has been reported to possess insecticidal activity against insects, including *S. frugiperda* (Taher et al., 2015; Vargas-Méndez et al., 2018). Ikawati et al. (2022) also reported insecticidal activity of clove oil and its major constituent eugenol against *Tribolium castaneum*, further supporting the insecticidal potential of clove-derived essential oil. In addition, Sitohang et al. (2024) reported insecticidal activity of *S. aromaticum* leaf oil containing 74.28% eugenol, with an LC_{50} of 0.58%. These findings support the potential contribution of *S. aromaticum* essential oil to the larvicidal activity observed in the present study.

The 1:2 formulation also showed relatively high toxicity, with LC_{50} and LC_{95} values of 0.16% and 2.27%, respectively. The insecticidal activity of *M. fragrans* essential oil has been documented and has been associated with its diverse volatile constituents (Ashokkumar et al., 2022; Cossetin et al., 2021). Therefore, the activity of the 1:2 formulation may reflect the contribution of bioactive constituents present in *M. fragrans* essential oil. However, specific compounds such as myristicin, safrole, or elemicin cannot be identified as responsible for the observed toxicity because the chemical composition of the oils in the present formulation was not determined.

The effectiveness of essential oil-based formulations against *S. frugiperda* has also been reported in previous studies. Lemus de la Cruz et al. (2022), for example, demonstrated larvicidal activity of a nanoemulsified *Cedrela odorata* essential oil formulation against *S. frugiperda*. Similarly, Lina et al. (2023) reported larvicidal activity of a nanoemulsion containing *Piper aduncum* and citronella oils against *S. frugiperda*. These studies support the potential of essential oil nanoemulsions as delivery systems for botanical insecticides, although their results cannot be directly compared with the present formulations because the essential oil species and formulation characteristics differed.

Overall, the 2:1 *S. aromaticum*: *M. fragrans* formulation exhibited the greatest larvicidal potency among the tested ratios based on its lowest LC_{50} and LC_{95} values. The result suggests that the relative

Table 2. Toxicity of nanoemulsion containing different ratios of *S. aromaticum* and *M. fragrans* essential oils against *S. frugiperda* larvae based on probit analysis at 96 hours after treatment

EO ratio (*S. aromaticum*:*M. fragrans*)	Intercept[a] ± SE[c]	Slope[b] ± SE[c]	LC_{50}[d] (CI[e] 50%) (%)	LC_{95}[d] (CI[e] 95%) (%)
1 : 1	1.14 ± 0.26	0.24 ± 0.70	0.28 (0.14–0.43)	7.74 (2.98–87.05)
2 : 1	1.46 ± 0.33	0.53 ± 0.11	0.10 (0.38–0.17)	1.42 (0.81–5.09)
1 : 2	1.44 ± 0.29	0.37 ± 0.88	0.16 (0.80–0.25)	2.27 (1.24–8.33)

[a]: intercept of probit regression line, [b]: probit regression slope, [c]SE: Standard Error. [d]LC: Lethal concentration, [e]CI: confidence interval.

proportion of the two essential oils is an important factor influencing larvicidal activity. The observed potency may reflect the combined contribution of bioactive constituents from both oils; however, the underlying chemical and biological mechanisms cannot be determined from the present data. Further studies involving chemical characterization of the essential oils and individual- or combination-compound assays are needed to clarify the constituents responsible for the observed activity.

Interaction Between *S. aromaticum* and *M. fragrans* Essential Oils. The interaction between *S. aromaticum* and *M. fragrans* essential oils was evaluated using the combination index (CI) at 96 hours after treatment (Table 3). The results showed that the type and magnitude of the interaction varied according to the mixing ratio and mortality level.

At the 1:1 ratio, the mixture exhibited weak synergism at LC_{50} (CI = 0.73) but antagonism at LC_{95} (CI = 2.10). This finding indicates that the interaction between the two essential oils was dependent on the level of biological response. The mixture enhanced toxicity at the concentration required to produce 50% mortality but became less effective than expected from the individual components at the higher mortality level. Such variation suggests that interactions among the constituents of the two essential oils may change with concentration. However, the specific biochemical mechanisms responsible for this shift cannot be determined from the present study.

The 2:1 formulation, containing a higher proportion of *S. aromaticum* essential oil, exhibited the strongest synergistic interaction, with CI values of 0.26 at LC_{50} and 0.32 at LC_{95}. This formulation also showed the lowest LC_{50} and LC_{95} values among the tested mixtures, indicating the greatest larvicidal potency. The strong interaction may be related to the complementary biological activities of constituents present in the two essential oils. Eugenol, a major constituent commonly associated with the biological activity of *S. aromaticum* essential oil, has been reported to possess insecticidal activity and to affect insect neural and enzymatic systems (Taher et al., 2015). Essential oil constituents of *M. fragrans*, including myristicin, safrole, and elemicin, have also been associated with insecticidal activity (Ashokkumar et al., 2022; Cossetin et al., 2021). Nevertheless, because the chemical composition of the essential oils used in the present study was not characterized, the contribution of individual compounds to the observed synergism cannot be established.

The 1:2 formulation also exhibited synergistic interactions at both LC_{50} and LC_{95}, with CI values of 0.46 for both endpoints. Although the interaction was weaker than that observed for the 2:1 formulation, the result indicates that increasing the proportion of *M. fragrans* essential oil did not eliminate the synergistic interaction between the two oils. The observed effect may reflect interactions among multiple constituents of the two essential oils rather than the action of a single compound. Previous studies have reported insecticidal activity of *M. fragrans* essential oil and its volatile constituents, supporting its potential contribution to the combined larvicidal effect (Ashokkumar et al., 2022; Cossetin et al., 2021).

The stronger synergism observed in the 2:1 formulation suggests that this ratio provided the most favorable interaction between the two essential oils under the conditions tested. The nanoemulsion system may also have facilitated the dispersion of the hydrophobic essential oils in the aqueous phase, potentially improving their contact with the target larvae. Similar research has demonstrated the potential of nanoemulsified essential oils as delivery systems for larvicidal applications against *S. frugiperda* (Lemus de la Cruz et al., 2022). However, because the present study did not directly compare nanoemulsified and non-nanoemulsified formulations, the contribution of nanoemulsification to the observed synergistic effect cannot be confirmed.

Overall, the 2:1 *S. aromaticum*: *M. fragrans* formulation showed the strongest synergistic interaction at both LC_{50} and LC_{95} and exhibited the greatest larvicidal potency among the tested combinations. These findings indicate that the 2:1 ratio

Table 3. Combined toxicity of *S. aromaticum* and *M. fragrans* essential oils against *S. frugiperda* based on LC_{50} and LC_{95} values after 96 hours of exposure

E.O ratio (*S. aromaticum*: *M. fragrans*)	Combination index		Effect at LC_{50}	Effect at LC_{95}
	LC_{50}	LC_{95}		
1:1	0.73	2.10	Weak synergism	Antagonistic
2:1	0.26	0.32	Strong synergism	Strong synergism
1:2	0.46	0.46	Strong synergism	Strong synergism

is a promising formulation for further investigation against *S. frugiperda*. Further studies incorporating chemical characterization of the essential oils, individual-compound assays, and mechanistic analyses are needed to determine the constituents and biological processes responsible for the observed synergistic interaction.

Inhibition of Egg Hatching. The effects of the essential oil nanoemulsions on *S. frugiperda* egg hatching are presented in Table 4. All tested formulations inhibited egg hatching relative to the untreated control. The nanoemulsion containing *S. aromaticum* essential oil at 1.00% inhibited egg hatching by 73.94%, while the *M. fragrans* formulation at 1.50% produced 72.84% inhibition. According to the classification of Arivoli & Tennyson (2013), both treatments showed high inhibition of egg hatching.

The 2:1 mixture of *S. aromaticum* and *M. fragrans* essential oils, applied at a total concentration of 1.00%, produced the highest inhibition of egg hatching, reaching 88.00%. This response was classified as very high inhibition, whereas no inhibition was observed in the control treatment. The greater inhibitory activity of the mixed formulation suggests that combining the two essential oils may enhance their ovicidal effects. However, because a formal combination index analysis was not performed for the egg-hatching assay, the observed response should not be interpreted as confirmed synergism.

Essential oils contain various volatile compounds that may interfere with insect development and survival at different life stages. *S. aromaticum* essential oil is commonly associated with eugenol, which has been reported to exhibit broad biological activity against insects (de Melo et al., 2023). Previous studies have suggested that essential oil constituents may affect embryonic development by altering the integrity of the egg surface or interfering with physiological processes required for successful hatching (Jeyakumar & Lawrence, 2020).

Similarly, *M. fragrans* essential oil contains a diverse range of volatile compounds, including monoterpenes and phenylpropanoids, which may contribute to its biological activity (Cossetin et al., 2021). Although the specific mechanism by which the tested formulation inhibited *S. frugiperda* egg hatching was not examined in the present study, essential oils from other plant species have been reported to cause embryonic damage and inhibit egg development. For example, essential oils from *Cymbopogon citratus* and *Illicium verum* have been reported to reduce egg hatching by affecting the chorion and physiological processes associated with embryonic development in *Musca domestica* (Passara et al., 2024).

The high inhibition of egg hatching observed with the 2:1 mixture indicates that this formulation may have activity against multiple developmental stages of *S. frugiperda*. Nevertheless, the different concentrations used for the single essential oil and mixed treatments should be considered when comparing their activities. Further experiments using equivalent concentrations and a formal interaction analysis would be necessary to determine whether the enhanced ovicidal activity of the mixture results from synergistic interactions.

Feeding Inhibition. The nanoemulsion treatments reduced the amount of corn leaf tissue consumed by *S. frugiperda* larvae compared with the untreated control (Table 5). The nanoemulsion containing *S. aromaticum* essential oil at 1.00% produced a feeding inhibition rate of 78.51%, while the *M. fragrans* formulation

Table 4. Inhibition of egg hatching by *S. aromaticum* and *M. fragrans* essential oils against *S. frugiperda*

Treatment	Concentration	Unhatched eggs (%)	Category
S. aromaticum	1.00%	73.94	+++ (high)
M. fragrans	1.50%	72.84	+++ (high)
Mix essential oils (2:1)	1.00%	88.00	++++ (very high)
Control	-	0.00	-

Table 5. Feeding inhibition activity of *S. aromaticum* and *M. fragrans* essential oils against *S. frugiperda* larvae

Treatment	Concentration	Feeding inhibition (%)
S. aromaticum	1.00%	13.45
M. fragrans	1.50%	59.30
Mixed essential oils (2:1)	1.00%	61.02
Control	-	0.00

at 1.50% produced an inhibition rate of 92.84%. The 2:1 mixture, applied at a total concentration of 1.00%, resulted in a feeding inhibition rate of 93.18%.

All tested formulations therefore showed considerable feeding-inhibitory activity against *S. frugiperda* larvae. However, direct comparisons among treatments should be interpreted cautiously because the *M. fragrans* treatment was tested at a higher concentration (1.50%) than the *S. aromaticum* and 2:1 mixed formulations (1.00%). In addition, the difference between *M. fragrans* alone (92.84%) and the 2:1 mixture (93.18%) was only 0.34 percentage points. Therefore, the numerical difference does not by itself demonstrate superior antifeedant activity of the mixed formulation, and statistical analysis is required to determine whether the treatments differed significantly.

The antifeedant activity of *S. aromaticum* essential oil may be associated with its volatile constituents, particularly eugenol and other terpenoid compounds. These compounds have been reported to affect insect behavior and may reduce feeding through effects on sensory perception or other physiological processes (Astuthi et al., 2012). Consistent with the present findings, *S. aromaticum* essential oil has also been reported to reduce feeding activity in *S. frugiperda* larvae (Barbosa et al., 2018).

Botanical extracts containing non-volatile secondary metabolites have likewise been reported to exhibit antifeedant activity against *S. frugiperda*. For example, Aprialty et al. (2021) reported feeding inhibition by extracts of *Calotropis gigantea* and *Crescentia cujete* and attributed the activity to secondary metabolites such as tannins, saponins, and flavonoids. However, these findings should not be directly extrapolated to the essential oils used in the present study because flavonoids and tannins are generally non-volatile compounds and are not typically major constituents of steam-distilled essential oils.

The antifeedant activity of *M. fragrans* essential oil may similarly be related to its volatile constituents, which have been reported to possess insecticidal and behavioral effects. Nevertheless, the contribution of specific compounds to the feeding inhibition observed in the present study cannot be confirmed because the chemical composition of the essential oils was not characterized.

The relatively high feeding inhibition observed for the 2:1 mixture is noteworthy, particularly because it was achieved at a lower total essential oil concentration than that used for the *M. fragrans* treatment. However, this observation should not be interpreted as evidence of synergism because feeding inhibition was not evaluated using a combination-index or other formal interaction analysis. The high feeding inhibition of the 2:1 formulation, together with its larvicidal and ovicidal effects, indicates its potential as a botanical formulation against *S. frugiperda*. Further studies using equivalent concentrations, appropriate statistical comparisons, and formal interaction analysis would be necessary to determine whether the combination provides an advantage over the individual essential oils.

Emulsion Particle Size and Polydispersity Index. The particle size and polydispersity index (PDI) of the essential oil formulations are presented in Figure 1. Considerable variation in mean particle size was observed among the three formulations. The *M. fragrans* formulation had the smallest mean particle size, measuring 68.3 nm (Figure 1B), followed by the 2:1 mixed formulation, with a mean particle size of 95.5 nm (Figure 1C). In contrast, the *S. aromaticum* formulation showed a substantially larger mean particle size of 414.3 nm (Figure 1A).

The differences in particle size among the formulations may be associated with the physicochemical characteristics of the essential oils and the formulation conditions. Droplet size in essential oil nanoemulsions is influenced by factors such as oil-phase composition, surfactant characteristics and concentration, oil-to-surfactant ratio, and emulsification conditions (Moradi & Barati, 2019; Wan et al., 2019; Wan et al., 2020). In particular, variation in the physicochemical characteristics of clove oil nanoemulsions has been reported under different formulation conditions (Haro-González et al., 2023). Therefore, the larger particle size observed in the *S. aromaticum* formulation may reflect differences in the interaction between its oil components and the emulsification system rather than the presence of a particular chemical group alone.

The type of surfactant is also an important factor affecting droplet formation and size. Tween 80 has been widely used in essential oil emulsion systems because of its ability to facilitate the dispersion and stabilization of oil droplets in aqueous media. Previous studies have shown that the effect of Tween 80 on droplet size may vary depending on the oil phase and formulation conditions (Wan et al., 2020; Nagaraju et al., 2021). Differences in droplet size reported among surfactants, including Tween 80, Tween 20, Tween 40, Tween 60, SDS, and Triton X-100, further indicate that surfactant performance is formulation-dependent

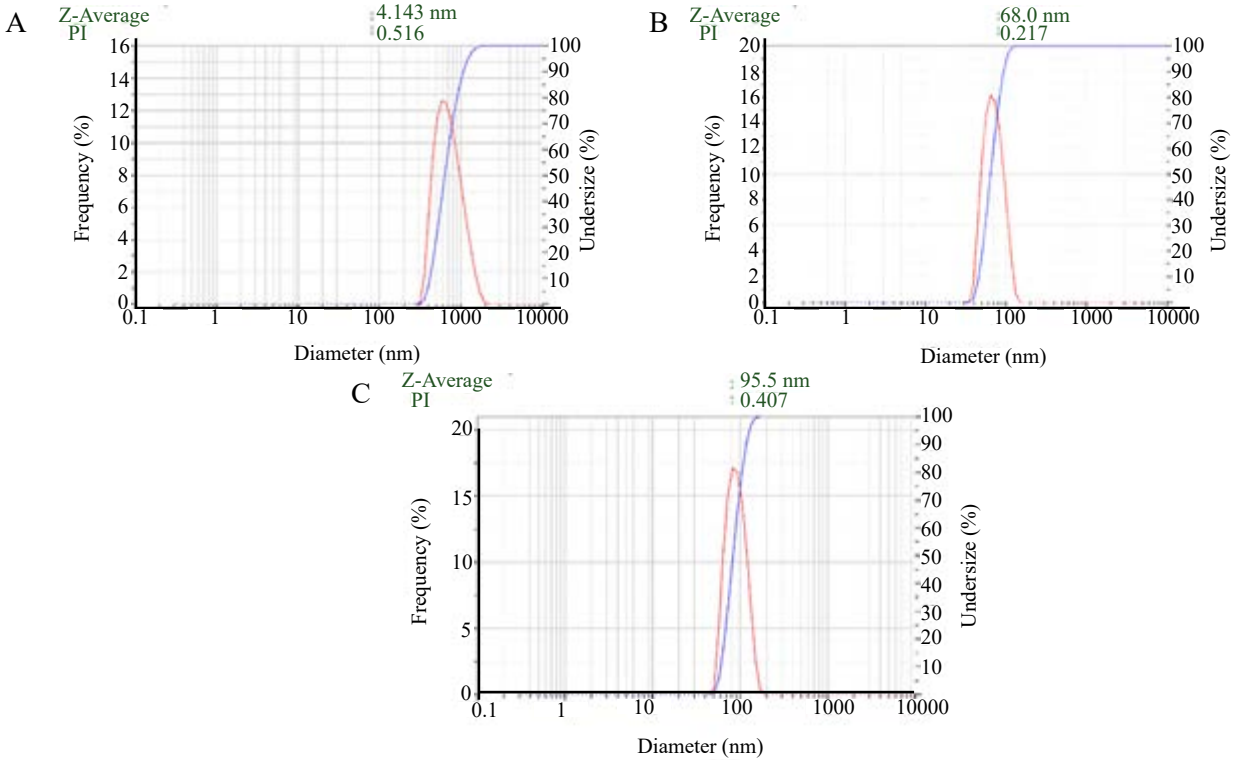

Figure 1. Particle size distribution of nanoemulsions. A. *S. aromaticum*; B. *M. fragrans*; C. Mixed *S. aromaticum* and *M. fragrans* (2:1).

rather than universally superior for all essential oil systems. Thus, the relatively large particle size of the *S. aromaticum* formulation in the present study may result from the specific characteristics of the oil–surfactant system used.

The PDI provides additional information on the uniformity of the droplet-size distribution. The *M. fragrans* formulation had the lowest PDI (0.279), indicating a relatively narrow and more uniform droplet-size distribution. A PDI below approximately 0.3 is generally considered indicative of a relatively homogeneous dispersion (Wu et al., 2012). In comparison, the *S. aromaticum* and 2:1 mixed formulations had PDI values of 0.516 and 0.407, respectively, indicating broader droplet-size distributions and greater polydispersity.

The combination of particle size and PDI suggests that the *M. fragrans* formulation had the most uniform dispersion among the tested formulations. However, these measurements should not be interpreted as direct evidence of long-term physical stability. Smaller droplets and a lower PDI can be associated with favorable dispersion characteristics and may reduce the tendency for gravitational separation or droplet aggregation (Ibrahim et al., 2015), but formulation stability should be confirmed through additional evaluations, including changes in particle size and PDI during storage, phase separation, and other relevant physicochemical parameters.

The relationship between particle size and biological activity is also noteworthy. The 2:1 mixed formulation, which had a mean particle size of 95.5 nm, exhibited the greatest larvicidal activity and the strongest synergistic interaction among the tested formulations. Its relatively small particle size may have facilitated dispersion of the formulation and increased the contact of bioactive components with the target larvae. Previous research has also demonstrated that botanical nanoemulsion formulations can exhibit both physicochemical characteristics and biological activity that are relevant to their potential as botanical pesticides (Nuryanti et al., 2018). Nevertheless, this relationship was not directly evaluated in the present study. Therefore, particle size should be considered a possible contributing factor to the observed biological activity rather than the sole determinant of insecticidal efficacy.

CONCLUSION

Three nanoemulsion formulations containing *S. aromaticum* and *M. fragrans* essential oils were successfully developed using a low-energy emulsification method via the phase inversion technique with Tween-80 as the emulsifier. Among the formulations, the 2:1 essential oil mixture

exhibited the best performance in terms of toxicity, egg hatching inhibition, and feeding inhibition activity, demonstrating a strong synergistic interaction. Physically, the 2:1 nanoemulsion had a small droplet size (95.5 nm) and an acceptable level of uniformity, making it the most promising composition for further development as a plant-based bioinsecticide for the control of *S. frugiperda.*

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AUTHORS' CONTRIBUTIONS

NSP conceived and planned the experiment. EW carries out maintenance and identification of *S. frugiperda*. EW collected observation data and prepared nanoemulsion test samples. DM and DB analyzed and interpreted the data. The author provided feedback and comments on the research flow, data analysis, interpretation, and the structure of the manuscript. All authors have read and approved the final manuscript.

COMPETING INTEREST

The authors declare that there are no conflicting interests concerning the publication of manuscripts.

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