

RESEARCH PAPER

## Characterization and bioactivity of nanoemulsions from *Leucaena leucocephala* and *Annona muricata* extracts against *Spodoptera frugiperda*

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### ABSTRACT

*Spodoptera frugiperda* is a major pest that attacks food and horticultural crops. The development of botanical pesticides in nanoemulsion form is one strategy to manage this pest. This research aimed to analyse the properties and effectiveness of nanoemulsions formulated using phase inversion and homogenization methods from *Leucaena leucocephala* and *Annona muricata* seed extracts against *S. frugiperda*. The research methods included mass rearing of *S. frugiperda*, extraction of *L. leucocephala* and *A. muricata* seeds, nanoemulsion formulation, and characterization of nanoemulsion properties. The results showed that the phase inversion method produced larger particle sizes compared to the homogenization method. Similarly, the phase inversion method resulted in a wider particle size distribution, while homogenization produced more uniform droplets. The nanoemulsion of the mixed extract of *L. leucocephala* and *A. muricata* seeds formulated using the homogenization method provided an antifeedant effect of 56.15% (medium inhibition criteria). The lowest antifeedant activity was observed in nanoemulsion from *L. leucocephala* using the phase inversion method (36.87%, low inhibition criteria). The highest ovicidal effect (72.93%) was also obtained from the mixed-seed nanoemulsion prepared by homogenization.

**Keywords:** Botanical pesticides, homogenization, phase inversion

### INTRODUCTION

The development of nanotechnology has progressed rapidly across multiple sectors, including agriculture (Wirawati et al., 2023). In the agricultural field, nanotechnology has been applied in the form of nano-fertilizers, nano-pesticides, soil improvement technologies, smart monitoring systems, and enhanced disease and pest management strategies. It also supports the production of value-added products derived from animal by-products and agricultural waste (Vijayakumar et al., 2022). Among these innovations, nano-pesticides offer notable advantages, particularly their ability to control pests over extended periods through slow and controlled release of active ingredients (Pradhan & Mailapalli, 2020). Their nanoscale particle size enhances the solubility and bioavailability of active compounds, enabling high efficacy even at low application rates. Such formulations have been shown to increase crop productivity while reducing inputs,

including labor and water use (Ngegba et al., 2022). These benefits highlight the importance of exploring potential botanical sources for the development of nano-biopesticides.

Corn is one of Indonesia's most important agricultural commodities, serving not only as a staple in several regions but also as a critical raw material for the feed and food industries (Jayadi & Latief, 2023). However, annual corn production can suffer yield losses of up to 50% due to infestations by the fall armyworm *Spodoptera frugiperda*, resulting in substantial economic losses for farmers (Herlinda et al., 2022). Infestations of *S. frugiperda* can even reach 100% (Djunaedy et al., 2024). This invasive pest is known for its aggressive feeding behavior on corn (Yang et al., 2022), and is highly polyphagous, attacking more than 350 plant species from 76 families, including various vegetables, grasses, and major field crops such as soybeans, sorghum, and rice (Chen et al., 2023).

Current efforts to control *S. frugiperda* still rely heavily on synthetic pesticides. However, excessive and improper use of synthetic pesticides leads to insecticide resistance, environmental contamination, harm to non-target organisms including beneficial insects, food and feed residues, and reduced biodiversity (Giuliano et al., 2024). These negative impacts underscore

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the urgent need for alternative pest management approaches, particularly the use of botanical pesticides as a component of integrated pest management (IPM). The development and adoption of botanical pesticides offer a promising strategy to reduce dependence on synthetic chemicals (Ngegba et al., 2022).

Botanical pesticides are derived from plant parts that function as toxicants, repellents, binding agents, or growth inhibitors of pests (Haritha et al., 2021). Their bioactive compounds commonly include secondary metabolites such as alkaloids, terpenoids, and phenolics (Latumahina et al., 2020). One promising botanical pesticide source is *Leucaena leucocephala*, a member of the Fabaceae family widely found in tropical regions, including Indonesia. This plant commonly grows in non-cultivated areas such as vacant land, roadsides, and home gardens, making it abundant and inexpensive. Its seeds contain mimosine, an alkaloid with toxic, antiproliferative, and apoptotic properties that inhibit mitosis, DNA and RNA replication, and protein synthesis (Kato-Noguchi & Kurniadie, 2022). Another example is *Annona muricata*, a tropical fruit plant extensively cultivated in Indonesia. Although the fruit is widely consumed, the seeds are typically discarded, representing a potential low-cost botanical pesticide resource. *A. muricata* seeds contain acetogenins and annonains, compounds known to act as stomach and contact poisons with insecticidal, larvicidal, repellent, and antifeedant effects (Naik & Sellappan, 2020).

One major challenge in the use of plant extracts as botanical pesticides is their low water solubility and instability under environmental conditions, which often leads to rapid degradation and reduced efficacy (Pavoni et al., 2020). Nanoemulsion-based formulations offer a promising solution to these limitations (Hadji et al., 2023; Nuryanti et al., 2023). Nanoemulsions are transparent emulsions with particle sizes ranging from 0.1 to 200 nm (Adak et al., 2020). Their nanoscale droplets enhance bioavailability and increase the biological activity, effectiveness, and efficiency of botanical pesticides (Sharma et al., 2020). Thus, nanotechnology provides a platform to overcome the inherent limitations of plant extracts and develop environmentally friendly, effective, and economically viable pest control solutions.

Nanoemulsions can be produced using either high-energy or low-energy techniques (Mustafa & Hussein, 2020). High-energy methods utilize mechanical devices such as microfluidizers, high-pressure homogenizers, and ultrasonicators, whereas low-energy methods include spontaneous emulsification, phase inversion, phase transition temperature, microemulsion dilution,

and classical emulsification (Safaya & Rotliwala, 2020). Several nanoemulsion formulations have been successfully developed for insect control. For example, *Cedrela odorata* essential oil nanoemulsion has proven effective against *S. frugiperda* (de la Cruz et al., 2022), while *Allium sativum* essential oil nanoemulsion exhibits toxic and antifeedant effects on *Spodoptera littoralis* larvae (Giuliano et al., 2024). Nanoemulsions of peppermint and eucalyptus essential oils have also demonstrated strong toxicity and enhanced effectiveness against *S. littoralis* (Shaker et al., 2022).

To date, research on nanoemulsion-based botanical pesticides for *Spodoptera* spp. has largely focused on assessing biological activity without evaluating which nanoemulsion preparation method yields the most effective pest control. Therefore, this study aims to compare the antifeedant and ovicidal effects of nanoemulsions produced using the phase inversion and homogenization methods from *L. leucocephala* and *A. muricata* seed extracts against *S. frugiperda*.

## MATERIALS AND METHODS

**Research Site.** This research was conducted at the Plant Production Laboratory 1, Lampung State Polytechnic, for nanoemulsion formulation and activity testing against *S. frugiperda*. Measurement of particle size and polydispersity index (PDI) was carried out at the Integrated Laboratory & Research Center, University of Indonesia (ILRC UI).

### Mass Rearing and Maintenance of *S. frugiperda*.

Larvae of *S. frugiperda* were collected from the practice garden of Lampung State Polytechnic and subsequently reared in the Tanaman 1 Laboratory. Third-instar larvae were transferred into separate containers at a density of five larvae per container to prevent cannibalism and were maintained until pupation. Pupae were placed in gauze-covered cages inside plastic jars lined with sterile sawdust. Emerging adults were fed a 10% honey solution dripped onto cotton and hung inside the cage.

Eggs laid by adult moths were transferred into plastic containers until hatching. The larvae used in this study were third-instar larvae, which represent the most suitable and manageable stage for experimental handling (Lina et al., 2023).

### Extraction of *L. leucocephala* and *A. muricata*

**Seeds.** Seeds of *L. leucocephala* and *A. muricata* were purchased from traditional markets in Bandar Lampung District. Seed extraction was conducted

using the maceration method. Air-dried seeds were peeled, and kernels were collected, ground using a grinder (Type-FFC23, 5 Kg capacity), and macerated in 99% methanol at a ratio of 1:5 (w/v) (Blandino et al., 2020). The mixture was filtered through Whatman No. 41 filter paper, and the filtrate was evaporated using a rotary evaporator (B-One® RE-2000B) at a 50 °C and 240 mbar until a thick extract was obtained (Nuryanti et al., 2023).

**Nanoemulsion Preparation.**

**Phase Inversion Method.** Nanoemulsions were prepared following Nuryanti et al. (2023) by adding the aqueous phase into the organic phase. Extract and Tween 80 (1:1, v/v) were homogenized using a magnetic stirrer at 750 rpm at temperatures below 29 °C. Distilled water was added slowly (4 mL/min) until the total volume reached 100 mL, followed by stirring for 30 min at 750 rpm.

**Homogenization Method.** The homogenization procedure followed Modarres-Gheisari et al. (2019) with slight modifications. Extract and Tween 80 (1:1, v/v) were mixed, and distilled water was added while stirring at 750 rpm until the total volume reached 500 mL. The mixture was then homogenized using a Wiggins D-500 homogenizer at 25,000 rpm and 30 °C for 20 min.

**Botanical Pesticide Nanoemulsion Formulation.** Nanoemulsions of single extracts were prepared using both the phase inversion and homogenization methods. Mixed-extract nanoemulsions were formulated using the better-performing method based on particle size analysis. Formulations followed Nuryanti et al. (2023).

The concentration of both single and mixed-extract nanoemulsions was set at 2% of the total solution. Extracts were mixed with Tween 80 (1:1, v/v) until homogeneous, followed by the addition of distilled water to reach a final volume of 100 mL. The formulation details are presented in Table 1.

**Nanoemulsion Characteristic.**

**Measurement of Particle Size and Polydispersity Index (PDI).** Particle size and PDI of the nanoemulsions were analyzed using a Horiba SZ-100 Particle Size Analyzer (PSA), applying the Dynamic Light Scattering (DLS) technique (Pratiwi et al., 2022).

**Antifeedant Effect.** The antifeedant activity of nanoemulsions was evaluated following Lina et al. (2023). Corn leaves were cut into 4 × 4 cm pieces and placed in containers with 10 third-instar *S. frugiperda* larvae. Leaves previously treated with nanoemulsion formulations were exposed to larvae for 24 hours. Leaf area consumption was determined by tracing leaves on millimeter paper; uneaten portions were shaded, and consumed areas were calculated.

The antifeedant index (AF) was calculated using the formula from Mokodompit et al. (2013):

$$AF = \frac{CI - TI}{CI} \times 100\%$$

AF = Antifeedant effect (%);

CI = Area of control leaves consumed (mm<sup>2</sup>);

TI = Area of treated leaves consumed (mm<sup>2</sup>).

Feeding inhibition criteria followed Mokodompit et al. (2013) (Table 2).

**Ovicidal Effect.** The ovicidal activity test was adapted from Ardiansyah et al. (2024) with modifications. *S. frugiperda* eggs on the surface of corn leaves were sprayed with nanoemulsion formulations at specified concentrations. Eggs were allowed to hatch, and the number of hatched eggs was recorded. Ovicidal activity (%) was determined using the formula from Harikarnpakdee & Chuchote (2018):

$$PP = \frac{NC - NT}{NC + NT} \times 100\%$$

PP = Ovicidal activity (%);

NC = Number of eggs hatched in the control group;

NT = Number of eggs hatched in the treatment group.

Table 1. Formulation of botanical pesticide nanoemulsion from *L. leucocephala* and *A. muricata* seed extracts

Nanoemulsion formulation	Method
<i>L. leucocephala</i> seed extract	Inversion phase Homogenization
<i>A. muricata</i> seed extract	Inversion phase Homogenization
Mixed extract of <i>L. leucocephala</i> dan <i>A. muricata</i> seed (1:1)	Best method

## RESULTS AND DISCUSSION

### Particle Size and Polydispersity Index (PDI).

Particle size analysis of nanoemulsions was performed using a Horiba SZ-100 Particle Size Analyzer (PSA) following Demisli et al. (2020) and Nuryanti et al.

Table 2. Antifeedant effect criteria of *S. frugiperda*

Antifeedant effect criteria	Antifeedant effect (FI)
High	$FI \geq 80\%$
Quite high	$60\% \leq FI < 80\%$
Medium	$40\% \leq FI < 60\%$
Low	$0\% \leq FI < 40\%$
No antifeedant effect	$FI = 0\%$

Source: Mokodompit et al. (2013).

(2023). Nanoemulsions are generally defined as dispersions with droplet diameters of 5–200 nm, surrounded by surfactant molecules that stabilize the system (Malode et al., 2022). Smaller particle sizes enhance penetration and absorption of bioactive compounds into insect tissues, increasing biological

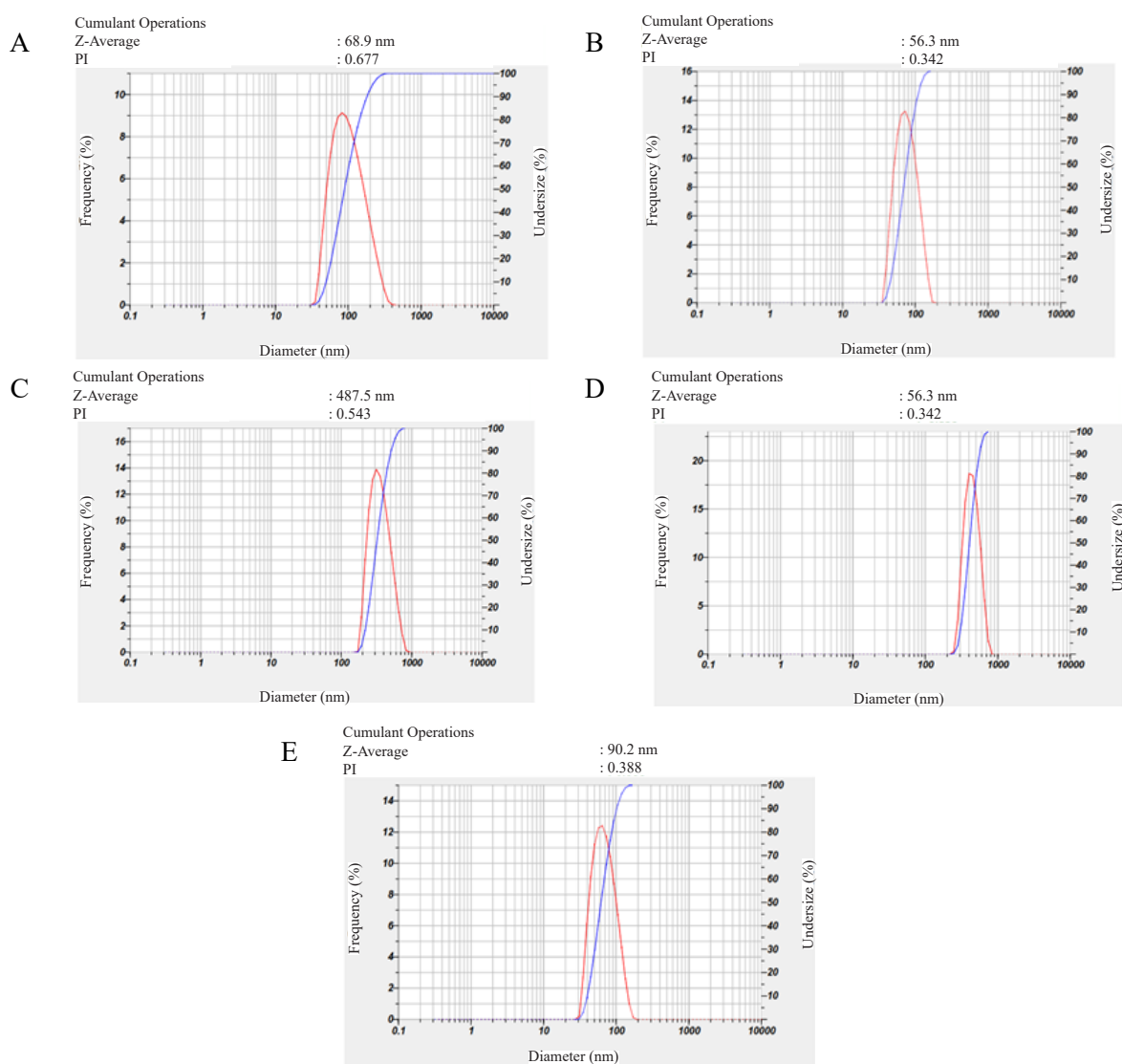


Figure 1. Results of nanoemulsion particle size analysis. A. *L. leucocephala* seed extract using the phase inversion method; B. *L. leucocephala* seed extract using the homogenization method; C. *A. muricata* seed extract using the phase inversion method; D. *A. muricata* seed extract using the homogenization method; E. Mixture of *L. leucocephala* and *A. muricata* seeds extracts.



activity (Pascual-Villalobos et al., 2019).

The PSA results are presented as distribution curves (Figures 1). The frequency (%) indicates the proportion of droplets at a given size, the red line shows the particle size distribution range, and the blue curve represents undersize accumulation. In addition to the Z-average droplet size, the PDI value is also generated by PSA. The smaller the PI value, the more homogeneous the particle size (Prihantini et al., 2019). PDI reflects size uniformity: values near 0 indicate homogeneous droplets (Sahumena et al., 2023). PDI values of 0.01–0.5 indicate a narrow distribution, 0.5–0.7 a medium distribution, whereas values >0.7 indicate broad or aggregated systems prone to sedimentation (Setianingsih et al., 2023).

**Nanoemulsion of *L. leucocephala* Seed Extract.** The phase inversion method produced a Z-average of 68.9 nm and PDI of 0.667 (Figure 1A). The particle size falls within the nanoemulsion criteria (<200 nm), but the PDI value indicates a medium distribution. Droplet sizes ranged from 30–130 nm, with a major peak at 80 nm (9%) and undersize droplets (<80 nm) accounting for 45%.

The homogenization method yielded smaller droplets (56.3 nm) and a more uniform PDI of 0.342 (Figure 1B). The nanoemulsion formed from *L. leucocephala* seed extract using the homogenization method can be classified as a nanoemulsion because the particle size is less than 200 nm (Adak et al., 2020). The PDI value also indicates a narrow particle size distribution (0.01–0.5), reflecting good droplet uniformity. Droplets ranged from 30–110 nm, with the largest peak at 70 nm (13.25%) and undersize droplets (<70 nm) reaching 50%. This confirms that homogenization improves droplet uniformity and reduces overall droplet size.

**Nanoemulsion of *A. muricata* Seed Extract.** Nanoemulsion of *A. muricata* prepared by phase inversion had a large Z-average of 487.5 nm and a PI of 0.543 (Figure 1C), indicating the formulation did not meet nanoemulsion criteria and exhibited medium uniformity. Droplet sizes ranged from 170–1000 nm, dominated by a 300 nm peak (14%) with 40% undersize.

Using homogenization, the droplet size decreased but remained above the nanoemulsion threshold (328.0 nm; PDI 0.590) (Figure 1D). The size range was 200–800 nm, with the highest peak at 400 nm (19%) and 45% undersize. These results suggest that compounds within *A. muricata* extract are more

difficult to reduce to nanoscale sizes, regardless of emulsification method.

**Mixed Seed Extract Nanoemulsion.** The mixed of *A. muricata* and *L. leucocephala* produced a Z-average of 90.2 nm and a PDI of 0.388 (Figure 1E). Both values indicate a stable, homogeneous nanoemulsion. Droplets ranged from 30–200 nm, with a main peak at 65 nm (12.5%) and 45% undersize. This suggests that combining extracts improves emulsification efficiency, possibly through synergistic physicochemical interactions.

**Comparison Between Methods: Phase Inversion vs. Homogenization.** Both emulsification methods produced different particle sizes and levels of uniformity. In both botanical extracts, homogenization consistently generated smaller droplets. For *L. leucocephala* seed extract, the phase inversion method produced a particle size of 68.9 nm, whereas homogenization yielded 56.3 nm. Similarly, for *A. muricata* seed extract, the phase inversion method produced a particle size of 485.7 nm, while homogenization resulted in 328.0 nm.

The polydispersity index (PDI) values also showed notable differences. The phase inversion method tended to produce a broader particle size distribution compared to homogenization, as reflected in the PDI values of *L. leucocephala* nanoemulsion (0.667 for phase inversion vs. 0.342 for homogenization). However, for *A. muricata* nanoemulsion, the phase inversion method produced a slightly smaller PDI value (0.543) compared to homogenization (0.590).

These findings align with Kotta et al. (2015), who reported that although phase inversion is a low-energy method, it can yield uniform droplets under optimized conditions.

**Mechanistic Interpretation.** There are two main approaches to developing nanoemulsions based on botanical pesticides: high-energy emulsification, such as homogenization, and low-energy emulsification, such as phase inversion. These methods produce nanoemulsions with different physicochemical properties, particularly with regard to particle size and polydispersity index (PDI). Studies on *L. leucocephala* and *A. muricata* seed extracts have shown that homogenization produces smaller particle sizes than phase inversion. For *L. leucocephala*, homogenization yielded a particle size of 56.3 nm, whereas phase inversion produced 68.9 nm. In *A. muricata*, the difference was more pronounced: 328.0 nm with homogenization compared to 485.7 nm with

phase inversion. Additionally, particle size distribution tends to be narrower in homogenized nanoemulsions, as indicated by smaller PDI values (e.g., 0.342 for *L. leucocephala*) relative to phase inversion (0.667).

However, the nature of the active ingredient greatly influences the final characteristics. For example, in *A. muricata*, the PDI value for phase inversion (0.543) was slightly smaller than the value for homogenization (0.590). This suggests that the effectiveness of each method is affected by the chemical and physical properties of the materials involved. Consistent with the findings of Kotta et al. (2015), phase inversion—despite being a low-energy method—can still produce stable and uniform nanoemulsions under optimized formulation conditions.

Homogenization methods utilize high energy, such as high pressure, mechanical shear, or ultrasonication, to break oil droplets in the continuous phase (usually water) into nanoparticles. This technique enables the formation of nanoemulsions with very small particles (typically <100 nm) and a uniform size distribution. Mechanical forces more effectively overcome the interfacial tension between oil and water phases (McClements, 2012; Kumar et al., 2019). Nanoemulsions produced using homogenization are generally more stable against coalescence, flocculation, and sedimentation because the smaller particle size slows the mechanisms of physical instability. Furthermore, homogenization is suitable for producing formulations with low surfactant concentrations while maintaining efficiency, making it more environmentally friendly and cost-effective (Pires et al., 2023).

In contrast, phase inversion is an example of low-energy emulsification. This technique relies on changes in composition or temperature to invert the oil and water phases, forming nanoscale droplets. Its main advantages include the absence of expensive equipment, simpler processing, and compatibility with heat- or pressure-sensitive active compounds—qualities common to many plant-derived bioactive molecules (Solans & Solé, 2012; Tadros et al., 2004).

However, phase inversion has limitations, including reduced control over droplet size and the need for more complex formulation optimization. Nanoemulsions produced via low-energy methods often exhibit larger particle sizes (>100 nm), higher PDI values, and greater susceptibility to environmental instability. Therefore, although phase inversion is more energy-efficient, additional investigation is needed to assess the long-term stability and performance of the resulting nanoemulsions.

Overall, nanoemulsions produced using high-energy methods such as homogenization offer advantages in achieving smaller particle sizes and more uniform size distributions. Low-energy methods, on the other hand, provide benefits in terms of processing simplicity and preservation of sensitive active compounds. The selection of the most appropriate method should be based on the characteristics of the active ingredient, the intended application, and the desired balance between cost and energy efficiency in the production process.

**Antifeedant Effect.** Nanoemulsions of *L. leucocephala* and *A. muricata* seed extracts exhibited antifeedant properties against *S. frugiperda*, as the percentage of feeding inhibition was greater than 25%. This aligns with Wahyuni & Yuliani (2023), who stated that an extract can be considered an active antifeedant if it produces more than 25% inhibition. The nanoemulsion of mixed *L. leucocephala* and *A. muricata* seed extracts produced using the homogenization method achieved a feeding inhibition level of 56.15%, categorized as medium inhibition, while the lowest inhibition (36.87%, low category) was recorded for the *L. leucocephala* seed nanoemulsion prepared using the phase inversion method (Table 3).

The results indicated that the mixed-extract nanoemulsion provided the highest antifeedant percentage, although its antifeedant category did not differ from that of the *L. leucocephala* and *A. muricata* nanoemulsions produced via homogenization. The mixed extract was more effective than the individual

Table 3. Results of feeding inhibition tests on *S. frugiperda*

Type of extract	Method	Antifeedant effect % $\pm$ SD	Antifeedant criteria
<i>L. leucocephala</i> seeds	Inversion phase	36.87 $\pm$ 11.28	Low
	Homogenization	42.50 $\pm$ 10.67	Medium
<i>A. muricata</i> seeds	Inversion phase	37.43 $\pm$ 11.94	Low
	Homogenization	49.83 $\pm$ 11.37	Medium
Mixture of <i>L. leucocephala</i> and <i>A. muricata</i> seeds	Homogenization	56.15 $\pm$ 7.67	Medium

extracts because the active compounds from *L. leucocephala* and *A. muricata* complement each other. *L. leucocephala* seeds contain secondary metabolites such as saponins, tannins, flavonoids, and alkaloids, all known to exhibit antifeedant activity (Adelia & Iskandar, 2020). Saputri et al. (2020) reported that the tannin content in *L. leucocephala* seeds can reduce larval feeding by inhibiting reverse transcriptase and DNA topoisomerase activity. When protease enzyme activity is inhibited, amino acid formation is reduced, preventing protein synthesis and ATP production. This energy deficiency eventually leads to larval mortality.

Meanwhile, *A. muricata* seed extract contains acetogenins, including annonain, as well as saponins, flavonoids, and tannins, which also function as antifeedants (Irmawati et al., 2023). Therefore, combining the two extracts enhances antifeedant performance, which is reflected in the highest feeding inhibition percentage observed in this study.

The antifeedant percentage of the nanoemulsion mixture of *L. leucocephala* and *A. muricata* seeds was higher than that reported by Wahyuni & Yuliani (2023), who tested a combination of *L. leucocephala* and *Carica papaya* leaf extracts against *S. litura* and obtained only 36% inhibition at 15% extract concentration. This indicates that botanical pesticides in nanoemulsion form are more effective in controlling *S. frugiperda*. Lina et al. (2021) also explained that mixtures of plant extracts formulated as nanoemulsions are more effective and efficient because they can achieve pest control at lower concentrations. Moreover, nanoemulsion-based botanical pesticides reduce the amount of raw material required compared to single-extract formulations, helping to address issues related to resource limitations and dependence.

**Ovicidal Effect.** Nanoemulsion of *L. leucocephala* seed extract and *A. muricata* seed extract demonstrated ovicidal properties. The highest ovicidal percentage was observed in the nanoemulsion treatment containing a mixture of *L. leucocephala* and *A. muricata* seed extracts, reaching 72.93%. This was followed by the nanoemulsion of *A. muricata* seed extract produced using the homogenization method (55.91%), *L. leucocephala* seed extract produced using the homogenization method (33.78%), *A. muricata* seed extract produced using the phase inversion method (26.11%), and the lowest was *L. leucocephala* seed extract (23.36%) (Figure 2).

*L. leucocephala* seed extract contains flavonoids, tannins, alkaloids, and steroids (Adelia & Iskandar, 2020). The flavonoid compounds in the extract can penetrate the egg chorion or larval cuticle and diffuse into internal tissues, where they selectively disrupt nerve ganglia. This interference affects the ecdysone hormone, which is essential for larval development. Flavonoids can also impair embryonic development, resulting in non-viable or weak larvae (Saputri et al., 2020).

Meanwhile, *A. muricata* seed extract contains acetogenins—such as annonacin—along with saponins, flavonoids, and tannins (Irmawati et al., 2023). These compounds can inhibit ovicidal processes by disrupting enzymatic activity within the digestive tract of developing embryos, leading to impaired growth and development (Djatkiko et al., 2023).

The ovicidal percentage of the mixed nanoemulsion extract was higher than that of each single-extract nanoemulsion. The combination of both seed extracts enhances ovicidal activity due to the synergistic effect of their active compounds.

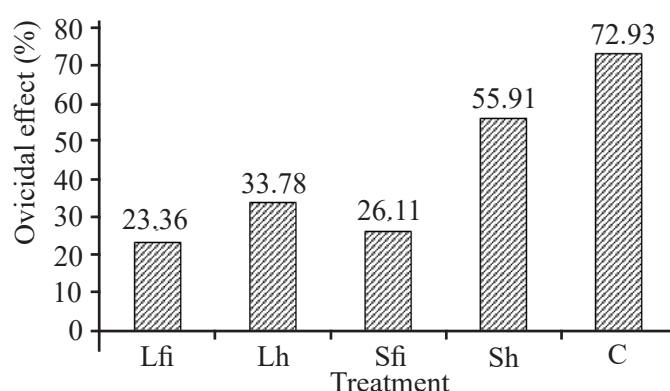


Figure 2. Results of the ovicidal effect test against *S. frugiperda*. Lfi = Nanoemulsion of *L. leucocephala* seed extract using the phase inversion method; Lh= Nanoemulsion of *L. leucocephala* seed extract using the homogenization method; Sfi= Nanoemulsion of *A. muricata* seed extract using the phase inversion method; Sh= Nanoemulsion of *A. muricata* seed extract using the homogenization method; C= Nanoemulsion of mixed extract of *L. leucocephala* and *A. muricata* seeds.

A mixture of *L. leucocephala* and *A. muricata* seed extracts provides a more complex profile of bioactive compounds compared to each extract alone, thereby strengthening the overall ovicidal effect (Luqman & Yuliani, 2023).

## CONCLUSION

The phase inversion method generally produced larger particle sizes and broader particle size distributions compared with the homogenization method, as indicated by higher polydispersity index (PDI) values. In contrast, homogenization resulted in smaller and more uniform nanoemulsion droplets. The nanoemulsion prepared from a mixture of *L. leucocephala* and *A. muricata* seed extracts using the homogenization method exhibited the strongest biological activity, with an antifeedant effect of 56.15% (medium inhibition criteria). The lowest antifeedant activity was recorded in the *L. leucocephala* nanoemulsion produced through phase inversion, with an inhibition rate of 36.87% (low inhibition criteria). The highest ovicidal effect (72.93%) was also observed in the mixed-extract nanoemulsion prepared via homogenization. These findings demonstrate that combining seed extracts in nanoemulsion form enhances both antifeedant and ovicidal activities against *S. frugiperda*, especially when processed using a high-energy homogenization method.

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## AUTHORS' CONTRIBUTIONS

NSP conceived and designed the experiment. SA conducted the maintenance and identification of *S. frugiperda*. NP collected observational data and prepared the nanoemulsion test samples. DM and DB performed data analysis and interpretation. All authors contributed to discussions on research design, data

interpretation, manuscript structure, and provided critical feedback. All authors read and approved the final manuscript.

## COMPETING INTEREST

The authors declare no competing interests related to the publication of this manuscript.

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