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Effectiveness of Nanoemulsion Formulated with Citronella and Garlic Essential Oils against Fall Armyworm (*Spodoptera frugiperda*) on Maize Plants

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Abstract

Fall armyworm (*Spodoptera frugiperda* J.E Smith) is a major invasive pest that threatens maize production in Indonesia. This study aimed to evaluate the activity of nanoemulsions of citronella and garlic essential oils, both individually and in combinations at ratios of 1:1, 2:1, and 1:2, against third-instar larvae of *S. frugiperda*. The essential oils were obtained by steam distillation and formulated into nanoemulsions using Tween 80. Toxicity was assessed via contact assays and analyzed with POLO-PC software to determine LC_{50} and LC_{95} values. The citronella–garlic ratio of 2:1 was the most promising, yielding the lowest LC_{50} (0.1 % at 96 hours) and consistently exhibiting strong synergistic effects at the LC_{50} level compared with the 1:1 and 1:2 ratios, which more frequently showed additive or antagonistic interactions. The citronella nanoemulsion displayed the smallest particle size (97.8 nm) but the highest polydispersity index (PI) (0.406), indicating lower physical stability. In contrast, the garlic nanoemulsion exhibited a particle size of 185.9 nm with a PI of 0.270, reflecting a more stable system. The citronella–garlic combination at a 2:1 ratio produced the largest particle size (204.9 nm) but the lowest PI (0.239), suggesting superior stability and a more uniform particle distribution. These findings support the potential of nanoemulsified essential oils as eco-friendly botanical insecticides.

Keywords: botanical insecticide; citronella; garlic; nanoemulsion; *Spodoptera frugiperda*.

INTRODUCTION

Fall armyworm (*Spodoptera frugiperda* J.E. Smith; Lepidoptera: Noctuidae) is a global invasive pest that causes severe damage, attacking more than 80 crop species, including maize, rice, and sorghum. Its aggressive biological traits such as high reproductive capacity, rapid life cycle, and wide mobility make it difficult to control. In Indonesia, FAW infestation has been reported to reduce maize yield by up to 50% if not managed properly (Saleh et al., 2023). According to FAO & CABI (2019), FAW infestations cause maize production losses ranging from 8.3 to 20.6 million tons annually, equivalent to an economic loss of US$ 2.5–6.2 billion.

To date, FAW management has largely relied on synthetic insecticides. However, overreliance on chemical insecticides has raised concerns regarding resistance development, environmental contamination, and risks to non-target organisms (Usseglio et al., 2023). This situation highlights the need for safer and more sustainable pest management approaches aligned with environmentally friendly agricultural practices (Giuliano et al., 2024). Botanical insecticides derived from essential oils are promising alternatives, as they contain bioactive compounds such as terpenoids and phenolics that act as larvicides, repellents, and feeding inhibitors (Gnankiné & Bassolé, 2017).

Citronella grass (*Cymbopogon nardus* L.) essential oil contains citronellal, geraniol, and citronellol, which have been shown to possess toxic and antifeedant properties against several insect pests (Saleh et al., 2023). Similarly, garlic (*Allium sativum* L.) essential oil, rich in allicin and diallyl disulfide, exhibits insecticidal activity against various Spodoptera species (Shang et al., 2019). A key limitation of essential oils lies in their volatile nature, but nanoemulsion technology can enhance their stability, solubility, and bioavailability while minimizing risks to non-target organisms (Gharsan, 2024).

Based on this potential, the present study aimed to evaluate the effectiveness of combined nanoemulsions of citronella and garlic essential oils in controlling *S. frugiperda*. The synergistic interaction of these essential oils in nanoemulsion form is expected to enhance insecticidal efficacy and provide a safer, sustainable alternative for pest management.

MATERIALS AND METHODS

Time and Place

The research was performed from June - December 2024. The formulation and toxicity tests of nanoemulsion were carried out at Plant Laboratory 1, Politeknik Negeri Lampung. The nanoemulsion characterization was conducted at the Integrated Laboratory and Research Center (ILRC), University of Indonesia.

Insect Rearing

Larvae of *S. frugiperda* were obtained from maize fields at Politeknik Negeri Lampung. The eggs were maintained on young maize leaves, and the larvae were continuously reared on baby corn and fresh leaves until reaching the third instar stage, which was subsequently used for all bioassay experiments.

Essential Oil Extraction

Plant materials used in this study were citronella grass (*C. nardus* L.; Poaceae) obtained from Pesawaran Regency, Lampung and garlic (Allium sativum L.; Amaryllidaceae) from Temanggung Regency, North Java Province. The plant materials were air-dried for 7 to 14 days, then chopped into small pieces and ground using an FF023-type blender to increase the surface area. Essential oils from each material were extracted by the steam distillation method (Puspa, et al 2017). During the distillation, water was heated to produce steam, which was passed through the plant materials in a distillation vessel. The steam carrying volatile oil components was condensed into liquid and separated from the water using a separating funnel. The essential oils obtained were stored in tightly sealed bottles and refrigerated at 4 ºC until further use for nanoemulsion formulation.

Nanoemulsion Formulation

Nanoemulsions were formulated using a low-energy emulsification technique through phase inversion. This method was adapted from Ostertag et al. (2012) with slight modifications, where the aqueous phase was slowly introduced into the organic phase. The formulations consisted of essential oils from *C. nardus* (citronella grass) and Allium sativum (garlic), combined with emulsifiers in the proportions described in Table 1. Each essential oil sample was mixed with an emulsifier and distilled water. The preparation began by placing the essential oil in a beaker, followed by the addition of Tween 80 as the emulsifier (Nuryanti et al., 2018). A magnetic stir bar was inserted and the beaker placed on a magnetic stirrer. Distilled water was then added dropwise at a rate of 4 mL/min under continuous stirring at 500 rpm. The resulting mixture was homogenized with a HG 150 homogenizer at 2500 rpm for 10 minutes. The emulsification process was conducted at room temperature (< 27 ºC).

Table 1. Formulation of nanoemulsion.

Formulation	Composition		
	Composisiton Essentials Oil	**Emulsifier**	**Aquadest**
Single	Citronella (2.00)	Tween 80 (2.00)	96.00
Single	Garlic (1.50)	Tween 80 (1.50)	97.00
Mixed 1 : 1	Citronella (1.00) + Garlic (1.00)	Tween 80 (2.00)	96.00
Mixed 2 : 1	Citronella (1.33) + Garlic (0.66)	Tween 80 (2.00)	96.00
Mixed 1 :2	Citronella (0.66) + Garlic (1.33)	Tween 80 (2.00)	96.00

Toxicity Assay

The toxicity assay was conducted to determine the Lethal Concentration (LC) values of the treatments. The test procedure followed the method of Nuryanti et al (2022) with modifications, using essential oils of citronella grass (*C. nardus*) and garlic (*A. sativum*) at mixture ratios of 1:1, 2:1, and 1:2 (Table 1). Each formulation was tested in three replicates across six concentration levels: 0%, 0.125%, 0.25%, 0.5%, 1%, and 2%. Treatments were applied by the contact method on groups of 10 third-instar larvae of *S. frugiperda*. Mortality observations were recorded at 24, 48, 72, and 96 hours after treatment (HAT). The LC values were calculated using probit analysis to determine the relationship between concentration levels and larval mortality response.

Compatibility Test of Mixed Extracts

The compatibility test of the extract mixture was based on the LC values of the single and mixed extracts. The LC values were used to determine the combination index (CI). The combination index indicates the type of interaction between the extract components. The calculation of the combination index (CI) was conducted using the formula proposed by Chou & Talalay (1984).

Emulsion Droplet Size and Polydispersity Index (PDI)

The droplet size and polydispersity index (PDI) of citronella and garlic essential oil nanoemulsions were determined using a Particle Size Analyzer (PSA) with the Dynamic Light Scattering (DLS) technique (Noor et al., 2015). For analysis, three drops of each nanoemulsion sample were diluted in 20 mL of distilled water to obtain an appropriate concentration and reduce multiple scattering effects. The diluted sample was transferred into disposable cuvettes and placed in the measurement chamber, with the detector positioned at a 90° angle relative to the laser beam. Measurements were performed using Zetasizer software by entering the solvent's refractive index, while laser intensity was automatically adjusted. As the laser passed through the sample, the light scattered due to the Brownian motion of the nano-droplets was detected. The recorded fluctuations were processed to calculate the average hydrodynamic diameter and particle size distribution. The droplet size was expressed in nanometers, while the PDI value indicated the distribution uniformity and stability of the nanoemulsion formulation.

RESULTS AND DISCUSSION

Toxicity of Citronella Essential Oil Nanoemulsions

The LC_{50} and LC_{95} values obtained showed a decreasing trend with longer exposure time. At 24 hours, LC_{50} was recorded at 1.01% and LC_{95} at 5.93%, whereas at 96 hours these values decreased to 0.65% for LC_{50} and 3.34% for LC_{95} (Table 2). This reduction in lethal concentration indicates that the toxic effectiveness of the essential oil increases over time, meaning that lower doses are required to achieve 50% and 95% mortality in the population. Furthermore, the 95% confidence intervals (CI 95%) for LC_{50} and LC_{95} also tended to narrow at longer observation times, indicating greater precision of the estimates as treatment duration increased.

Table 2. Estimation of Probit Analysis Parameters for Citronella Essential Oil against *S. frugiperda.*

Essential Oils	HAT^a	a^b±SE	b^c±SE^d	LC^e_{50} (CI^f 95%) (%)	LC_{95} (CI 95%) (%)
Citronella	24	2.14 ± 0.33	0.17 ± 0.11	1.01 (0.81-1.33)	5.93 (3.66-13.03)
	48	2.44 ± 0.35	0.22 ± 0.12	0.81 (0.67-1.01)	3.84 (2.62-6.93)
	72	2.30 ± 0.33	0.25 ± 0.11	0.70 (0.51-1.02)	3.64 (2.06-11.69)
	96	2.32 ± 0.33	0.26 ± 0.11	0.65 (0.53-0.81)	3.34 (2.29-6.00)

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.

This study is in line with Kotambunan et al. (2020), who reported that citronella grass (*C. nardus*) extract was able to kill C. pavonana larvae from the first day of observation, with mortality rates increasing in accordance with higher extract concentrations applied to the feed. Citronella essential oil has an LC_{50} value of 0.36% against Mizus persicae (Dono et al., 2019). The findings of this research show that citronella extract applied by the contact method demonstrated increasing toxic effectiveness, as indicated by decreasing LC_{50} values of 1.01% at 24 HAT, 0.81% at 48 HAT, 0.70% at 72 HAT, and 0.65% at 96 HAT.

Citronella (*C. nardus*) contains essential oils with insecticidal properties that can suppress reproductive ability and feeding activity in insects. The essential oil consists of various active compounds such as citral, citronellal, geraniol, myrcene, nerol, farnesol, methyl heptenol, and dipentene. Among these, citronellal (about 33.06%) and geraniol (28.40%) are the main constituents responsible for its contact toxicity (Bayala et al., 2020).

Toxicity of Garlic Essential Oil Nanoemulsions

The analysis using Polo-PC software showed that garlic essential oil nanoemulsion exhibited toxic effects against *S. frugiperda* during the observation period from 24 to 96 hours after treatment (HAT). Based on the data presented in Table 3, there was a gradual decrease in both LC_{50} and LC_{95} values with increasing exposure time. At 24 HAT, the LC_{50} value was recorded at 0.99% and LC_{95} at 5.86%. At 48 HAT, LC_{50} decreased to 0.78%, while LC_{95} dropped to 5.16%. This downward trend continued at 72 HAT, with LC_{50} at 0.68% and LC_{95} at 4.60%. In the final observation at 96 HAT, LC_{50} and LC_{95} reached their lowest levels at 0.66% and 4.11%, respectively. The decline in LC values indicates that the toxicity of the garlic nanoemulsion against *S. frugiperda* larvae progressively increased over time.

Table 3. Estimation of Probit Analysis Parameters for Garlic Essential Oil against *S. frugiperda.*

Essential Oils	HAT^a	a^b±SE	b^c±SE^d	LC^e_{50} (CI^f 95%) (%)	LC_{95} (CI 95%) (%)
Garlic	24	2.13 ± 0.37	0.28 ± 0.14	0.99 (0.79-1.37)	5.86 (3.42-15.02)
	48	2.01 ± 0.33	0.28 ± 0.11	0.78 (0.55-1.30)	5.16 (2.50-27.04)
	72	1.99 ± 0.32	0.29 ± 0.10	0.68 (0.54-0.89)	4.60 (2.79-10.53)
	96	2.07 ± 0.32	0.30 ± 0.10	0.66 (053-0.85)	4.11 (2.579-8.82)

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

This study is consistent with Giuliano et al. (2024), which reported that nanoformulations based on garlic essential oil exhibited high toxicity against second-instar larvae of *S. littoralis*. Mortality data fitted the probit model, and the calculated LC (Lethal Concentration) values confirmed that the formulation's effectiveness mainly acted as a larvicide within the first 24 hours after application. The essential oil concentrations required to kill 50% and 90% of the larvae within 24 hours were 1.72% and 2.79%, respectively. Furthermore, Firmansyah et al. (2023) investigated the effectiveness of garlic extract against *Spodoptera litura* on chili plants. The results showed that garlic extract significantly increased larval mortality, reduced the frequency and intensity of pest attacks, and positively affected fruit number and weight. The LC_{50} and LC_{90} values in laboratory tests were 19.66% and 29.97%, respectively, while the median lethal time (LT_{50}) at 32% concentration was 3.37 days.

The use of garlic as a natural pesticide also improves plant health because its extract contains various active compounds such as allicin, alliin, saltivine, scordinin, and menteilalin trisulfide (Soetomo, 1987). These compounds possess insecticidal properties and function as repellents, since allicin—responsible for garlic's characteristic odor—is disliked by insects.

Toxicity of Citronella and Garlic Essential Oil Formula to *S. frugiperda* Larvae.

The bioassay results of citronella and garlic essential oils against third-instar *S. frugiperda* larvae demonstrated that the 2:1 (w:w) mixture consistently produced a strong synergistic effect at both LC_{50} and LC_{95} levels from 24 to 96 HAT, while other ratios showed weaker or inconsistent synergistic and antagonistic interactions (Table 4). This finding suggests that the 2:1 nanoemulsion of citronella and garlic essential oils represents the most effective formulation. At both LC_{50} and LC_{95} levels, the interaction profile of the citronella–garlic nanoemulsion exhibited shifts in synergistic activity over time, reflecting changes according to the observation period. Such variability in the activity of botanical pesticide mixtures may be influenced by factors such as concentration, exposure duration, and the mode of action of insecticidal compounds at their target sites (Wulansari et al., 2022). Mixed formulations of botanical insecticides often show synergistic potential because of their enhanced effectiveness against pests. Combining essential oils not only allows a reduction in application dose but also helps to suppress the ability of pests to develop resistance (Russianzi & Prijono, 2019). The synergistic interaction is further supported by the presence of different classes of chemical compounds that together contribute to increased toxicity (Nuryanti, 2019).

Table 4. Toxicity of citronella and garlic essential oil formula to *S. frugiperda* Larvae.

E.O Ratio *A.sativum* *C. nardus*	HAT[a]	a[b]±SE	b[c]±SE[d]	LC[e]50 (CI[f] 95%) (%)	LC_{95} (CI 95%) (%)
1 : 1		1.32 ± 0.26	0.19 ± 0.71	0.72 (0.53-1.06)	12.65 (5.50-63.63)
1 : 2	24	1.50 ± 0.27	0.20 ± 0.75	0.66 (0.50-0.90)	8.14 (4.15-27.28)
2 : 1		1.22 ± 0.27	0.28 ± 0.75	0.20 (0.11-0.29)	4.58 (2.25-19.78)
1 : 1		1.45 ± 0.27	0.20 ± 0.73	0.60 (0.45-0.82)	8.14 (4.07-28.84)
1 : 2	48	1.37 ± 0.26	0.21 ± 0.72	0.50 (0.36-1.68)	7.87 (3.83-30.38)
2 : 1		1.22 ± 0.28	0.32 ± 0.81	0.14 (0.07-0.22)	3.31 (1.70-13.48)
1 : 1		1.44 ± 0.26	0.21 ± 0.72	0.51 (0.38-0.69)	7.13 (3.64-24.38)
1 : 2	72	1.38 ± 0.26	0.23 ± 0.72	0.41 (0.29-0.55)	6.36 (3.22-22.69)
2 : 1		1.33 ± 0.30	0.39 ± 0.92	0.13 (0.06-0.19)	2.23 (1.26-7.03)
1 : 1		1.37 ± 0.26	0.22 ± 0.71	0.43 (0.31-0.59)	6.87 (3.42-25.32)
1 : 2	96	1.47 ± 0.27	0.25 ± 0.75	0.36 (0.26-0.48)	4.75 (2.60-1402)
2 : 1		1.78 ± 0.40	0.89 ± 0.16	0.10 (0.04-0.14)	0.84 (0.57-1.77)

Noted: A[a]= Hour After Treatment, b[b] = intercept of probit regression line, c[c] =probit regression slope, SE[d]: Standard Error. [e]LC: Lethal Concentration, [f]CI: confidence interval.

The research results showed that all citronella and garlic essential oil mixtures tested via contact method were toxic to third-instar *S. frugiperda* larvae (Table 2). Based on the results, the 2:1 ratio of citronella to garlic consistently showed the lowest LC_{50} values across all observation times, indicating the highest level of toxicity. At 24 hours of exposure, the LC_{50} value for the 2:1 ratio was 0.20%, which was markedly lower than the 1:1 and 1:2 ratios. A similar trend was observed at 48 and 72 hours, where the LC_{50} values decreased to 0.14% and 0.13%, respectively. The lowest LC_{50} value was recorded at 96 hours for the 2:1 ratio (0.10%), demonstrating that this formulation was the most effective in killing *S. frugiperda* larvae.

According to Juniarti et al., (2010), a compound can be categorized as active or toxic when its LC_{50} value is below 1000 ppm or equivalent to 0.1%. Based on the results obtained, the citronella–garlic oil mixture at a 2:1

ratio proved to be the most effective compared to the 1:1 and 1:2 ratios, as shown by the lowest LC_{50} value of 0.10% at 96 hours of exposure. Citronella oil is known to contain citronellal, geraniol, citronellol, and limonene, which exhibit insecticidal and repellent activities mainly through interference with the insect nervous and olfactory systems (Nerio et al., 2010). In contrast, garlic oil is rich in organosulfur compounds such as allicin and diallyl disulfide, which can disrupt enzyme activity, respiration, and nerve signal transmission in insects (Chaubey, 2011).

The higher proportion of citronella metabolites in the 2:1 mixture likely contributed to its greater toxicity. The effectiveness of botanical insecticides generally increases when different essential oils are combined, since their chemical constituents act through multiple mechanisms, such as acute poisoning, feeding deterrence, repellency, inhibition of growth, or suppression of detoxification enzymes (Jiang et al., 2012). In this formulation, citronellal and geraniol play roles as neurotoxic and repellent agents, while allicin reinforces toxicity by impairing enzymatic and metabolic pathways. This combination of actions imposes more than one type of physiological stress on the larvae, leading to faster mortality and reducing the risk of resistance development. Tak & Yoon, (2022) also reported that terpenoid compounds like citronellal and geraniol can interfere with the central nervous system of insects, while sulfur compounds from garlic are effective in suppressing detoxifying enzymes. This complementary effect explains why the citronella–garlic nanoemulsion in a 2:1 ratio displayed the strongest larvicidal activity against *S. frugiperda*.

Compatibility Mixtures of Essential Oils of Lemongrass and Garlic.

The interaction characteristics between citronella and garlic extracts at various ratios were aimed at evaluating the potential for synergistic, additive, or antagonistic effects when the two extracts are combined in a single treatment. These interactions were analyzed based on the LC_{50} and LC_{95} values of the combinations, compared to the LC_{50} and LC_{95} values of each extract individually. Three mixture ratios were tested in this study, namely 1:1, 1:2, and 2:1 (citronella : garlic).

Table 5. Compatibility mixtures of citronella dan garlic essential oils against *S. frugiperda*.

Ratio Lemongrass : Garlic	HAT[a]	Combination Index Value		Mixed criteria	
		LC_{50}	LC_{95}	LC^{b}_{50}	LC^{b}_{95}
1:1	24	0.85	3.30	Additive	Antagonistic
	48	0.90	2.68	Additive	Antagonistic
	72	0.88	2.51	Additive	Antagonistic
	96	0.76	2.72	Weak Synergistic	Antagonistic
1:2	24	0.76	1.81	Weak Synergistic	Antagonistic
	48	0.72	2.39	Weak Synergistic	Antagonistic
	72	0.68	2.04	Weak Synergistic	Antagonistic
	96	0.68	1.61	Weak Synergistic	Antagonistic
2:1	24	0.21	0.91	Strong Synergystic	Additive
	48	0.18	0.91	Strong Synergystic	Additive
	72	0.20	0.64	Strong Synergystic	Weak Synergistic
	96	0.16	0.25	Strong Synergystic	Strong Synergistic

Noted: A[a]= Hour After Treatment, b[b] = intercept of probit regression line, c[c] =probit regression slope.

The results presented in Table 5 indicate that the combination of essential oils from citronella (*C. nardus*) and garlic (*A. sativum*) produced varying levels of insect mortality depending on the mixing ratio and duration of exposure. The interaction between these essential oil extracts was significantly influenced by their ratio. At a 1:1 ratio, the combination index (CI) at LC_{50} values tended to approach 1 (0.85–0.90), indicating an additive effect, although at LC_{95}, the interaction was more frequently antagonistic. This suggests that equal proportions of both oils do not necessarily enhance efficacy and may even weaken each other's effects at higher concentrations.

At a 1:2 ratio (citronella:garlic), the interaction yielded mild synergism at LC_{50} but remained antagonistic at LC_{95} up to 96 hours post-treatment. This indicates that garlic-dominated formulations were not sufficiently effective to induce strong synergistic action against *Spodoptera frugiperda* mortality. In contrast, the 2:1 ratio (citronella:garlic) resulted in consistently low LC_{50} values (0.21–0.16) and strong synergistic interactions across all observation periods. LC_{95} values also showed a tendency toward synergism, particularly at 72 and 96 hours. This aligns with the toxicokinetic-toxicodynamic theory proposed by Ashauer et al (2011), which states that prolonged exposure can enhance the cumulative toxic effects of active compounds. Therefore, this ratio can be considered the most promising formulation for development as a botanical insecticide, as the dominance of citronella appears to enhance garlic's toxic activity, resulting in greater combined efficacy than either oil alone.

Phytochemically, citronella oil contains major components such as citral, geraniol, and limonene, which are known to disrupt insect nervous systems, inhibit feeding behavior, and damage cellular membranes (Plata-Rueda et al., 2017). Garlic contains allicin, a volatile sulfur-containing compound that can induce or inhibit phase II detoxification enzymes in target organisms (Hasrianda & Setiarto, 2022). However, when both oils are combined at certain ratios, the interaction between their active constituents may become competitive, leading to antagonism—particularly at high concentrations required to achieve LC_{95}. A similar phenomenon was reported by Hasheminya & Dehghannya, (2020), who noted that essential oil combinations do not always exhibit synergistic effects but are highly dependent on the balance of active compounds and their respective mechanisms of action. These findings reinforce the concept that essential oil-based insecticides require precise formulation ratios to achieve maximum effectiveness. The combination with citronella-dominance was shown to be more effective, achieving insect mortality rates above 95%, compared to equal or garlic-dominated mixtures.

This is consistent with a review by Sarri et al. (2024), which emphasizes that interactions between essential oils are highly complex and may be synergistic, additive, or antagonistic, depending on concentration and compositional ratios. Synergistic effects can arise from the combination of different chemical classes that potentiate each other's toxic activity (Nuryanti et al., 2018). Thus, the citronella-garlic essential oil combination, particularly at a 2:1 ratio, holds significant potential for development as an environmentally friendly alternative for controlling agricultural insect pests such as *Spodoptera frugiperda*, a species known for its resistance to many chemical insecticides.

Emulsion Particle Size

Botanical pesticides are considered a promising alternative solution to replace synthetic pesticides, which often have negative impacts on the environment and human health. Essential oils from plants such as *C. nardus* L. (lemongrass) and *A. sativum* (garlic) are known to possess insecticidal, bactericidal, and fungicidal properties. Nevertheless, the main challenge associated with the direct application of essential oils lies in their volatile nature, poor water solubility, and instability in the presence of light and oxygen. Therefore, the formulation of nanoemulsions is regarded as a strategic approach to enhance the bioefficacy and stability of essential oils by reducing particle size and providing a homogeneous distribution, thus improving their potential as effective antimicrobial agents (Yakoubi et al., 2021). Tween 80, as a non-ionic surfactant, plays a crucial role in stabilizing these systems by reducing the interfacial tension between oil and water and facilitating the formation of stable nanodroplets (Yu et al., 2012).

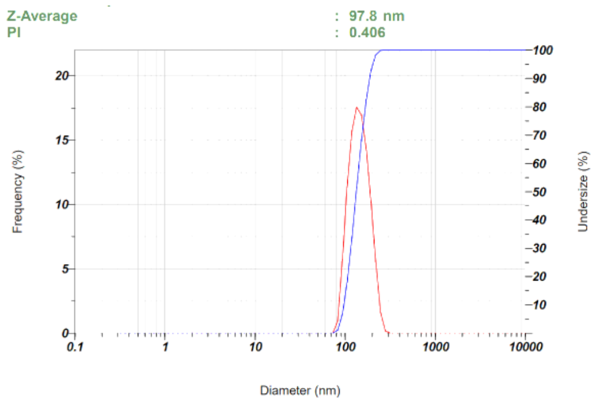

Figure 1. Particle size of lemongrass essential oil nanoemulsions.

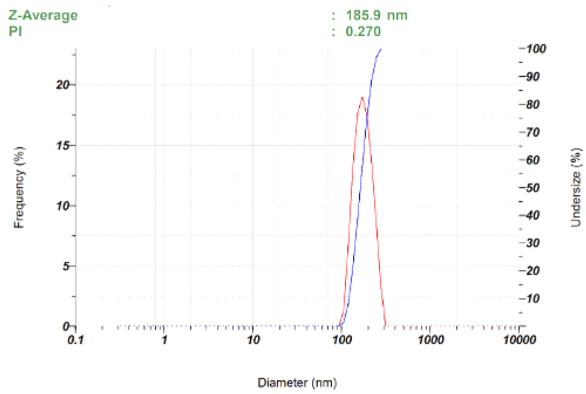

Figure 2. Particle size of garlic essential oil nanoemulsions.

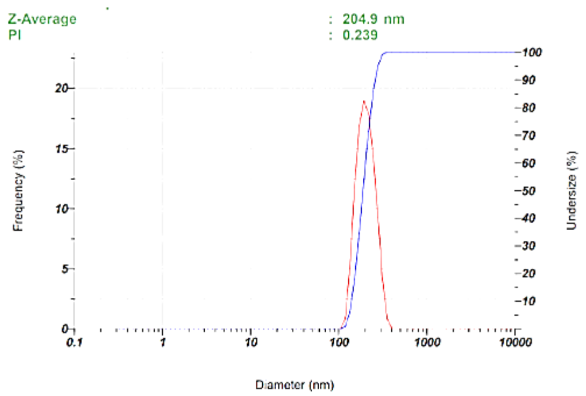

Figure 3. Particle Size 2:1 mixed essential oil nanoemulsions.

Results from the Particle Size Analyzer (PSA) indicate that all three samples exhibited monodisperse particle size distributions, reflecting good homogeneity within the emulsion systems. Figure 1, representing a nanoemulsion of lemongrass essential oil, showed an average particle size (Z-Average) of 97.8 nm with a polydispersity index (PI) of 0.406, indicating small particle size but lower physical stability. In contrast, Figure 2, representing garlic essential oil, displayed an average particle size of 185.9 nm and a PI of 0.270, indicative of a more stable system suitable for long-term storage. Meanwhile, Figure 3, a combination of lemongrass and garlic essential oils in a 2:1 ratio, exhibited the largest average particle size at 204.9 nm but demonstrated the best stability, with a PI of 0.239. A PI value below 0.3 is indicative of a narrow particle size distribution and high emulsion stability.

The chemical composition of each essential oil significantly influences the characteristics and biological potential of the formulations. Lemongrass contains active compounds such as citronellal and geraniol, which act as repellents and neurotoxins against insects (Du et al., 2015). On the other hand, garlic is rich in organosulfur compounds such as allicin, which exhibit bactericidal and fungicidal properties by inhibiting protein synthesis and disrupting the cell membranes of pathogens (Bhatwalkar et al., 2021). Through the nanoemulsion formulation, these two essential oils can act synergistically: the mixed nanoemulsion offers a dual mechanism combining the repellent action of garlic and the toxic effect of lemongrass, potentially preventing pest resistance and broadening the spectrum of control against insects, bacteria, and fungi. In field applications, the nano-sized particles of all three formulations enable more efficient penetration into plant leaf surfaces and target tissues, as well as increased contact efficacy with insects or pathogens. Furthermore, nanoemulsions are easily miscible with water, do not require additional organic solvents, and exhibit greater resistance to environmental degradation, making them an ideal technology for the development of safe, effective, and sustainable botanical pesticides.

Characterization results demonstrate that nanoemulsion formulations of essential oils, both as single components and in combination, hold significant potential as botanical pesticides. Lemongrass essential oil excels in terms of small particle size, albeit with lower stability. Garlic essential oil demonstrates a balance between particle size and stability. The third sample, a combination of lemongrass and garlic essential oils, exhibits the best physical stability and the greatest potential for synergistic effects, positioning it as a leading candidate for modern, multifunctional, and highly efficacious botanical pesticide formulations in sustainable agriculture.

CONCLUSIONS

Based on the results, it can be concluded that the nanoemulsion mixture of citronella (*C. nardus*) and garlic (*A. sativum*) essential oils is effective as a botanical insecticide against third-instar larvae of *Spodoptera frugiperda*. The 2:1 formulation proved to be the most promising, as it showed the lowest LC_{50} value (0.10% at 96 hours) and consistently exhibited strong synergistic effects at the LC_{50} level compared to the 1:1 and 1:2 ratios, which more frequently displayed additive or antagonistic interactions. This effectiveness is supported by the presence of citronellal, geraniol, and citronellol in citronella oil, which act as repellents and neurotoxins, as well as allicin in garlic oil, which disrupts enzymatic systems and insect metabolism. Together, these compounds provide dual effects by inhibiting feeding and accelerating mortality. Therefore, the 2:1 citronella–garlic nanoemulsion has strong potential to be developed as an eco-friendly botanical insecticide for controlling *S. frugiperda* and to support sustainable agriculture by reducing reliance on synthetic insecticides.

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