18

Egypt. J. Chem. **Vol. 69**, No. 2. pp. 245 - 253 (2026)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Preparation of Citronella and Thyme Essential Oil Nanoemulsions and Their Insecticidal Efficiency against Mosquitoes (*Culex pipiens*)

Nada A. Abdelmawla[1,*], Samia M. M. Mohafrash[1], Asmaa Z. El Sharkawy[2], Doaa S. Farghaly[2], Abdel-Tawab H. Mossa[1,*]

[1]*Pesticide Chemistry Department, Chemical Industries Research Institute, National Research Centre, 33 El Bohouth Street (former El Tahrir St.), P.O. 12622, Dokki, Giza, Egypt.*

[2]*Zoology and Entomology Department, College of Science, Al-Azhar University, P. O. 11765, Cairo 11865, Egypt*

Abstract

The objective of this study was to develop eco-friendly insecticides utilizing nanotechnology. Essential oils from citronella and thyme were extracted and analyzed using gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis of citronella oil identified Z-Citral and E-Citral as the main chemical constituents, comprising 34.45% and 42.99% of the oil, respectively. In contrast, o-Cymene and Thymol accounted for 28.44% and 26.84% of thyme oil, respectively. A nanoemulsion of these oils was formulated and assessed for its efficacy against mosquitoes, Culex pipiens. The nanoemulsions were prepared using essential oil and Tween 80 in varying ratios under ultrasonic emulsification for durations of 2.5, 5, and 10 minutes. Our findings indicate that the droplet size of the nanoemulsion is influenced by the emulsification parameters. The stable nanoemulsion exhibited droplet sizes of 20.2 nm for citronella and 54.6 nm for thyme. The results demonstrated that both thyme and citronella essential oils exhibit significant adulticidal and knockdown effects on Culex pipiens. The citronella and thyme oils showed KC_{50} values for knockdown at 0.424 and 5.651 mg/m^3 for essential oils and 10.954 and 6.977 mg/m^3 for their respective nanoemulsions. Furthermore, the adulticidal LC_{50} of citronella and thyme oils against Culex pipiens after 24 hours was recorded as 6.038 and 5.619 mg/ml for essential oils, and 20.245 and 11.048 mg/ml for their nanoemulsions. The findings suggest that nanoemulsions are formulated utilizing aqueous mediums, which are typically less toxic and more environmentally sustainable compared to traditional solvents. This attribute improves their safety for human use and promotes their application across various environments. Consequently, these eco-friendly nanoformulations can be regarded as viable alternatives to synthetic insecticides for the management of public health pests and vector-borne diseases.

Keywords: Citronella oil, green pesticides, Culex pipiens, Nanoemulsion, Thyme oil.

1. Introduction

Worldwide, mosquitoes (Culex pipiens) are recognized as significant vectors of various diseases, contributing to substantial health issues, social instability, economic detriment, and millions of fatalities annually [1, 2]. The challenges associated with treating vector-borne diseases are exacerbated by the limited efficacy of vaccinations and medications. Consequently, insecticides are deemed essential and effective instruments for mitigating disease transmission by managing vector populations. The emergence and widespread occurrence of pyrethroid resistance in mosquito populations, due to target-site mutations and enhanced detoxification mechanisms, complicates the management of these infections [3, 4]. The resurgence or proliferation of vector-borne diseases in certain regions may be linked to this resistance, which the World Health Organization (WHO) identifies as a significant threat to controlling mosquito-borne diseases. Despite not being used commercially until 1980, pyrethroid insecticides accounted for over 25% of the global market [5]. Recent studies have highlighted the significant toxicity of pyrethroid insecticides to mammals, as these chemicals have been shown to induce alterations in physiological activity and various pathological traits [6, 7].

Natural insecticides present a compelling alternative to synthetic options for managing mosquito populations. EOs such as lemon, eucalyptus, lavender, cinnamon, and citronella have shown remarkable effectiveness in repelling mosquitoes. Furthermore, the use of EOs as natural insecticides for pest control, particularly through nano formulations, has gained traction in the field of green pest management [8]. These eco-friendly pesticides, recognized for their low to negligible toxicity to mammals, are designed to target multiple toxic action sites in pests, ensuring high selectivity and minimizing the likelihood of resistance development [9, 10].

A comprehensive literature survey indicates that essential oils (EOs), including lemongrass, cedar, citronella, eucalyptus, geranium, cinnamon, clove, tea tree, basil, black seed, sage, thyme, orange, peppermint, neem, and soybean, exhibit significant insecticidal and repellent properties [8, 11].

*Corresponding author e-mail: abdeltawab.mossa@yahoo.com ; (A. H. Mossa).

Received Date: 15 July 2025, Revised Date: 23 August 2025, Accepted Date: 24 August 2025

DOI:10.21608/EJCHEM.2025.404022.12043

For example, lemon eucalyptus oil can provide over 95% protection for up to three hours. Likewise, cinnamon oil is effective not only in killing mosquito eggs but also in repelling adult mosquitoes. Neem oil, another potent natural insecticide, can be applied directly to the skin or used in sprays to deter these pests. Additionally, cultivating mosquito-repellent plants like marigolds, lavender, and citronella grass can create a natural barrier against mosquitoes. The emerging trend of nano formulation effectively reduces evaporation, thereby safeguarding EOs from degradation and extending the longevity of their residual effects. This innovative approach facilitates a straightforward application process and ensures a controlled release of the essential oils [12].

This study develops a new formulation of environmentally sustainable nanotechnology derived from Citronella and Thyme. The biological efficacy of the essential oils (EOs) and their respective nanoformulations were assessed against *Culex pipiens*. Furthermore, the chemical composition of the EOs was meticulously analyzed using Gas Chromatography-Mass Spectrometry (GC/MS).

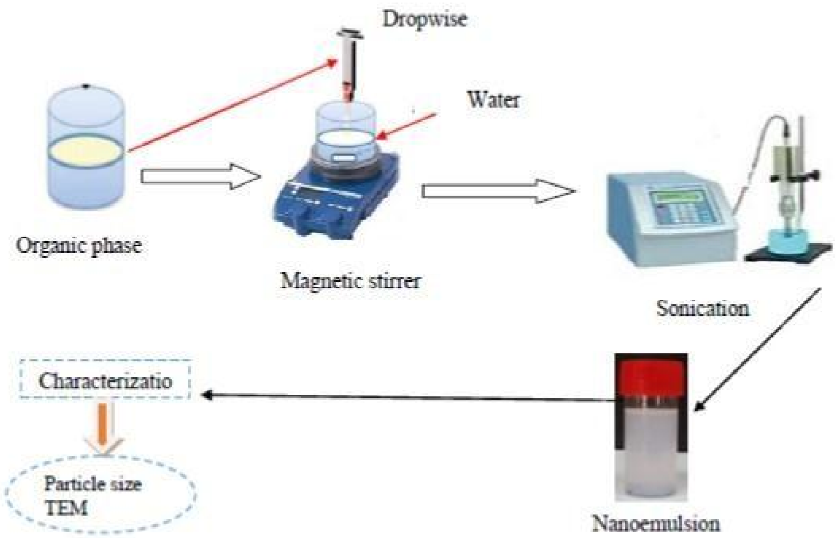


Figure 1: A schematic representation of the preparation for nanoemulsion and characterization process.

2. Materials and methods

Chemicals and reagents

The 99.8% ethanol was purchased from Fisher Technology, a German company. LOBA CHEMIEPVT.LTD provided a nonionic surfactant, Polysorbate 80 (Tween 80 Extra Pure). The remaining chemicals of reagent grade were acquired from businesses with scientific wholesalers in Cairo, Egypt.

Essential oils

In collaboration with the pressing and extraction unit of natural oils at The National Research Centre, Dokki, Giza, the essential oils (EOs) used in this study were extracted from a few chosen plants during the ripening period.

Gas chromatography-mass spectrometry analysis

For GC/MS analysis, a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer) in conjunction with a chromatograph TRACE GC Ultra Gas (THERMO Scientific Corp., USA) was employed (Department of Medicinal and Aromatic Plants Research, National Research Centre). A TG-5MS column (30 m x 0.25 mm i.d., 0.25 µm film thickness) was used with the GC/MS. The carrier gas, helium, had a split ratio of 1:10 and a flow rate of 1.0 mL/min. The temperature program started at 60 °C for one minute, increased by 3 °C per minute to 240 °C, and was maintained there for one minute while the injector and detector's temperatures were maintained at 240 °C. By employing a spectral range of m/z 40−450 and electron ionization at 70 eV, MS was produced.

Identification of essential oil constituents

In this investigation, mass spectra (genuine chemicals, Wiley spectral library collection, and National Institute of Standards and Technology (NIST) library) were used to identify most of the compounds in EO.

Formulation and characterizations of EO nanoformulations

Nanoemulsion preparation

According to Mossa et al. [9, 10], with a few minor modifications, citronella and thyme EOs were combined with deionized water and Tween 80, a non-ionic surfactant, to form oil-in-water nanoemulsions.

Nanoemulsion physicochemical and stability studies

According to Mossa et al. and Golemanov et al. [15, 16], stability tests including centrifugation, heating, thermodynamics, cooling, and freezing cycles of nanoemulsion had taken place in accordance with accepted procedures. Samples were examined for stability following each physicochemical and stability test [17, 18]. The stable samples in this investigation were assessed for droplet size dispersion. For additional stability measurements, all stable samples were stored in a lab setting for four weeks.

Nanoemulsion characterization

The dynamic light scattering apparatus, which is a 23 °C, 632.8 nm line of a HeNe laser with a 90° angle for droplet size, was used to scan the stable nanoemulsion samples. The tiny droplets discovered in the samples were analyzed using a transmission electron microscope (TEM) (model JEM-1230, Jeol, Tokyo, Japan). A grid of carbon-coated copper was loaded with the sample after it had been diluted (1:10) with distilled water (D.W.). The phosphotungstic acid solution at 2% (pH = 6.7) was used to stain the slide before imaging.

Bioassays

Insect colony

The susceptible strains of *Culex pipiens* that came from the Medical Insects Research Institute, Ministry of Health, Dokki, Giza, Egypt, were colonized in a laboratory setting with 26 ± 1 ℃, 12 hours of light and 12 hours of darkness, and 60–70% relative humidity. To meet the mosquito requirements for bioassays, *Culex pipiens* adults were also raised in the National Research Center's lab in accordance with the Kauffman Standard Method [13] and Sawicki *et al.* [14].

The larvae of the tested mosquito were reared in white pans that are 35-40 cm in diameter and 10cm deep and they contain about 1.5 litres of dechlorinated water. Larvae were provided with tetramine, which is a tropical fish food for the development of larvae and fecundity of females.

Knockdown concentrations (KC)

The knockdown activity was studied using the standard technique [19]. Experiments were conducted in the chamber (1 m³). Fifty adult female mosquitoes, ranging in age from three to five days, were let loose in the space. The plants' essential oils were allowed to evaporate using vaporizing equipment, "electrical." Based on the findings of the first screening, oils were evaluated at 1.25, 2.5, 5, 10 and 20 mg /m³. The number of adult female mosquitoes killed was counted after 10, 30 and 60 minutes. The humidity was maintained at 60-80% R.H., and the temperature was maintained at 26 ± 2.0 ℃. The knocked concentration (KC_{50}) was recorded with an average of three replicates. KC_{50} was calculated using probit analysis. Control was carried out by exposing the mosquitoes to the vapors of deodorized kerosene (DOK).

Adulticidal assay

The standard WHO technique for assessing susceptibility [20, 21] was used to assess the adulticidal activity of citronella and thyme essential oils and their nanoemulsions against adult *Culex pipiens* using WHO tubes and impregnated paper. Red and green spots were used to identify the tubes containing the treatments and control samples, respectively. The tubes with the red dots contained pieces of Whatman® (No. 1) filter paper (12 cm × 15 cm) that had been treated with 1 ml of *Citronella and Thyme* oils or nanoemulsions at varying concentrations. Filter paper and one milliliter of water were placed in the control tubes, which were identified by their green dots. The attached movable slide was then used to blow 25 mosquitoes into the control tubes lined with sterile white paper after they had been collected using aspirators. The slide was opened, the treated paper was placed within the treatment tubes, the mosquitoes were gently blown into the tubes, and the slide was then closed once more. Every treatment and control procedure were finished in five replicates. The results of mortality were recorded after 24 hours of exposure and used as an endpoint.

Statistical analysis

The data was analyzed using the Statistical Package for Social Sciences (SPSS 26.0 for Windows) program. The probit analysis was used to determine the lethal concentrations (LC_{50}) and the knockdown concentrations (KC_{50}). The mortality percentage was determined using Abbott's formula [22], and Finney *et al.* [23] determined the toxicity levels.

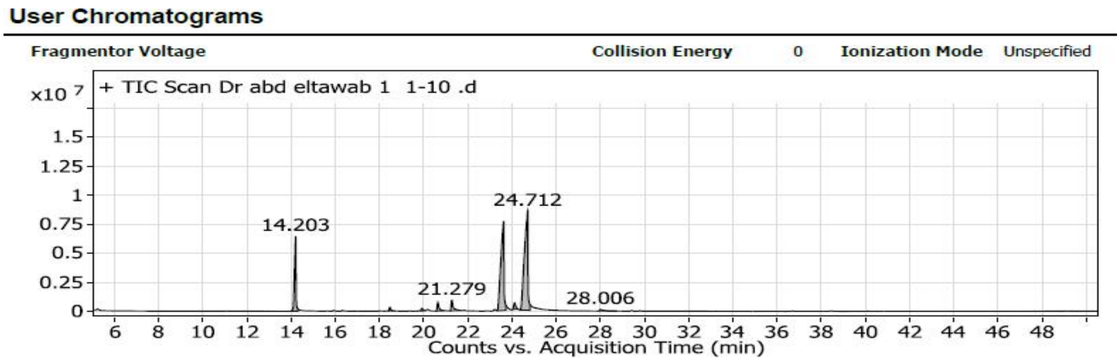
Table 1: GC/MS analysis of citronella oil.

Peak	RT	Chemical compounds	Area %
1	14.203	beta.-Myrcene	14.1
2	18.47	Linalool	0.76
3	19.921	6-Octenal,7-methyl-3-methylene-	0.53
4	20.644	Isoneral	1.92
5	21.279	Isogeranial	2.72
6	23.622	Z-Citral	34.45
7	24.118	Geraniol	1.56
8	24.712	E-Citral	42.99
9	28.006	2,6-Octadien-1-ol,3,7-dimethyl-,acetate,(Z)-	0.98
		Total	99.03

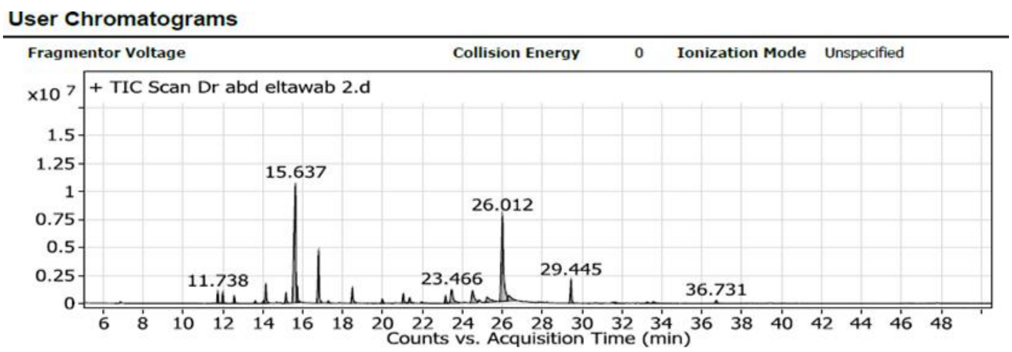
Table 2. GC/MS analysis of thyme oil.

Peak	RT	Chemical compounds	Area %
1	11.738	Bicyclo[3.1.0]hex-2-ene,4-methyl-1-(1-methylethyl)-	1.58
2	11.994	Alpha-pinene,(-)-	1.44
3	12.565	Camphene	1.03
4	13.62	(-)-.beta.-Pinene	0.37
5	14.017	1-Octen-3-ol	0.45
6	14.151	Beta Myrcene	2.95
7	15.159	Alpha-Terpinolene	1.5
8	15.637	o-Cymene	28.44
9	15.724	(S,E)-2,5-Dimethyl-4-vinylhexa-2,5-dien-1-ylacetate	1.88
10	16.797	Gamma-Terpinene	8.35
11	17.275	Cis-sabinene hydrate	0.35
12	18.487	Linalool	2.62
13	19.985	Camphor	0.69
14	21.04	endo-Borneol	1.56
15	21.355	Terpinen-4-ol	1.16
16	21.955	Alpha-Terpineol	0.33
17	23.156	Thymyl methyl ether	1.23
18	23.466	Z-Citral	4.61
19	24.512	2,6-Octadienal,3,7-dimethyl-,(E)-	4.3
20	25.243	Anethole	2.7
21	26.012	Thymol	26.84
22	29.445	Caryophyllene	3.71
23	31.666	Aromadendrene	0.33
24	36.731	Caryophylleneoxide	0.77
		Total	99.19

RT: Retention Time

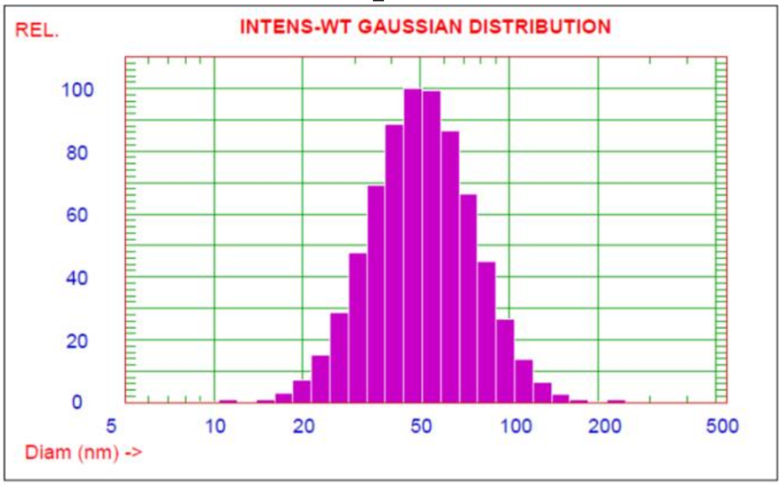


(a)

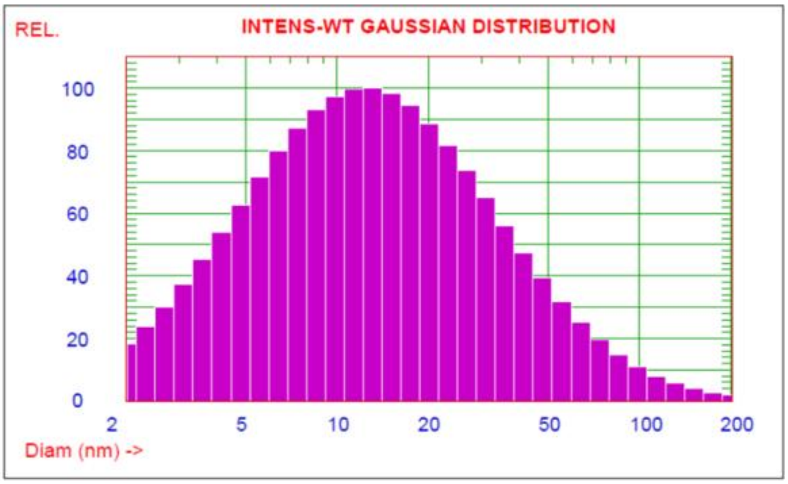


(b)

Figure 2: Citronella (a) and thyme (b) oils GC chromatogram.



Sample no.2B3 of citronella oil (20.2 nm)



Sample no.3C3 of thyme oil (54.6 nm)

Figure 3. Particle size distribution of citronella nanoemulsion sample no. 2B3 (20.2 nm) and thyme nanoemulsion sample no. 3C3 (54.6 nm).

Table 3. Code of prepared nanoemulsion samples with the ratio of essential oils, Tween 80, and deionized water (w/w).

Sample no.	Essential oil	Sample Code	Ratio (oil: Tween 80, w/w)	Formulation (g/100 g)		
				Oil	Tween 80	D.W
1	Citronella	A	2:1	5	5	90
		B	1:1	5	7.5	87.5
		C	1:2	5	2.5	92.5
2	Thyme	A	2:1	5	5	90
		B	1:1	5	7.5	87.5
		C	1:2	5	2.5	92.5

D. W. =Distilled water.

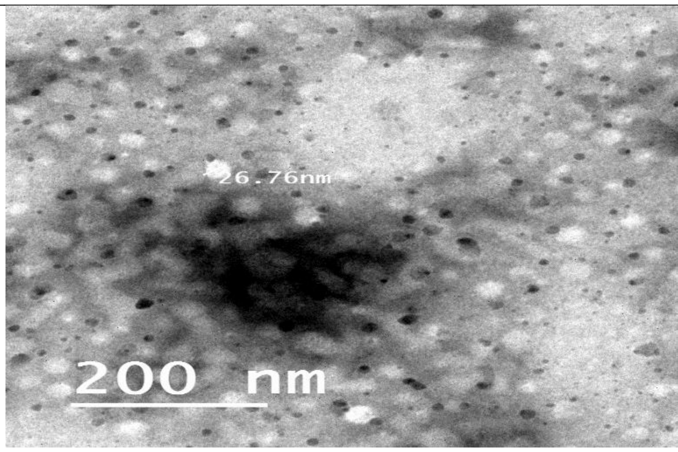


Figure 4. Transmission electron microscopy (TEM) of droplets of citronella nanoemulsion sample no. 2B3.

Table 4. Stability and physicochemical studies of the prepared nanoemulsions along with their droplet sizes (X = failed, √ = passed).

Sample Code	Sonication time (min)	Ratio (oil: Tween 80, w/w)	Centrifugation	Heating–Cooling cycle	Freeze–Thaw cycle	Result	Droplet size (nm)	Variance (P.I.)
A1	2.5	2:1	-	-	-	X	-	-
B1	5	1:1	-	+	+	X	-	-
C1	10	1:2	+	+	+	√	20.2	0.972
A2	2.5	2:1	-	-	-	X	-	-
B2	5	1:1	-	+	+	X	-	-
C2	10	1:2	+	+	+	√	54.6	0.162

Table 5. Knockdown concentrations of citronella and thyme essential oils against adult *Culex pipiens* after 10, 30, and 60 minutes of exposure:

Treatment	KC_{50} (mg/m^3)	Confidence limits		KC_{90} (mg/m^3)	Confidence limits		Chi-square (*df*=3)	*P* value
		Lower limit	Upper limit		Lower limit	Upper limit		
After 10 minutes								
Citronella	11.55704	8200.60	14385.66	39.04017	30115.69	64033.53	1.88	0.39
Thyme	7.19823	6428.86	7924.48	11.78996	10906.79	12899.81	0.59	0.74
After 30 minutes								
Citronella	.55433	7456.41	4115.96	24.12191	19747.15	34305.05	0.57	0.90
Thyme	2.82597	1008.33	1768.09	3.21931	323304.76	5095.67	0.79	0.37
After 60 minutes								
Citronella	.42474	5421.85	3052.17	10.87587	9314.21	13501.75	2.04	0.15
Thyme	5.65195	2016.67	3536.19	6.43862	646609.48	10191.33	0.79	0.37

KC_{50}, concentration induced 50% Knockdown. Probit analysis is completed by SPSS 26.0 for Windows.

Table 6. Toxicity of citronella and thyme essential oils and their nanoemulsions against the adult of *Culex pipiens* after 24 h of exposure:

Treatment	LC_{50} (mg/ml)	Confidence limits		LC_{90} (mg/ml)	Confidence limits		Chi-square (*df*=3)	*P* value
		Lower limit	Upper limit		Lower limit	Upper limit		
Normal emulsion								
Citronella	6.03806	4896.71	7061.73	13.55224	12237.98	15276.28	2.62	0.105
Thyme	5.61985	4671.57	6471.89	11.40237	10371.75	12719.12	5.96	0.051
Nanoemulsion								
Citronella	20.24539	17715.59	23212.43	38.51952	33812.46	45631.83	1.62	0.203
Thyme	11.04899	7025.92	14402.67	37.01849	31476.37	46042.57	1.45	0.228

LC_{50}, concentration induced 50% mortality. Probit analysis is completed by SPSS 26.0 for Windows.

3- Results and Discussion

The chemical compositions of the essential oils were ascertained in this study using GC-MS analysis, and the outcomes were contrasted with the data from the Wiley Mass Spectral Library. The GC-MS analysis of citronella oil indicates that its primary chemical constituents are E-Citral and Z-Citral, which account for 42.99% and 34.45% of the oil, respectively (Table 1 and Figure 2). The proportions of o-Cymene and thymol in thyme oil were 28.44% and 26.84%, respectively (Table 2 and Figure 2). The distinctive lemon aroma of citronella oil is attributed to its two primary ingredients, Z-Citral and E-Citral [24, 25].

Citronella oil's components, which include 10.82% citronellol, 20.07% geraniol, and 36.11% citronellal, were identified by Harianingsih *et al.,* [26] using GC-MS. Additionally, another study revealed that citronella oil had 80.65% p-menthane-3,8-diol and 75.95%citronellol, 76.63% geraniol, and 95.10% citronellal. It would also be advantageous to consider variables that affect the precision and dependability of GC-MS analysis. Methods for sample preparation, equipment calibration, and reference library selection can all affect how accurate the results are. Moreover, minor variations in the composition of essential oils might result from regional or environmental variances in plant growth, which can impact their chemical profile and bioactivity [27].

The study found that EOs had strong adulticidal action against *Culex pipiens* after 24 hours, with lethal concentrations (LC_{50}) of 6.038 mg/ml and 5.619 mg/ml for citronella and thyme, respectively. As a result, these oils were utilized in ultrasonic emulsification to create nanoformulations (Figure 1). To find the optimal ratio, Table 3 shows the various ratios of oil (5%) and Tween 80, a hydrophilic surfactant, by (1:1, 1:2, 2:1 w/w). A 5% concentration of oil, deionized water, and tween 80 were used to create nanoemulsion, which was then subjected to varying sonication periods of 2.5 and 5 minutes.

To ascertain the stability of nanoemulsions, physicochemical processes such as centrifugation, freeze-thaw cycles, and heating-cooling cycles were studied (Table 4). The nanoformulations of thyme oil sample 3C3 and citronella oil sample 2B3 show good stability. The others exhibit different degrees of separation stages and creaming, indicating instability. For droplet size distributions, the stable formulations that were produced were therefore employed.

Citronella oil (20.2 nm) and thyme oil (54.6 nm) are droplet sizes for stable nanoemulsions in the current study, sample no. 2 B3 and sample no. 3C3, respectively (Figure 3). Nanodroplet homogeneity is measured using the index of polydispersity (PI), which ranges from 0.0 to 1.0. Additionally, the transmission electron microscopy (TEM) image shows good dispersion and spherically shaped droplets in nanoformulations (Figure 4). The findings showed a relationship between the oil, surfactant ratio, and sonication times and nanoemulsions with smaller droplets. According to previous study, suitable nano emulsion droplet sizes fall between 20 and 200 nm. Balance between the oil and surfactant's Hydrophile-Lipophile Balance (HLB) value produced a good nanoemulsion with smaller droplets [28, 29].

Balancing various factors is crucial when creating nanoformulations to produce stable nanoemulsions with smaller droplets. This influences the mobility of hydrophilic substances through Ostwald ripening or molecular diffusion. As a substance's solubility in a droplet increases through the continuous phase due to increased interfacial curvature, the Kelvin-Thomson effect causes larger droplets to grow at the expense of smaller ones. Therefore, previous studies indicated that oil, sonication durations, and surfactant ratio all affect the nanoemulsions with smaller droplets [28, 29].

This study tested the efficacy of essential oil concentrations of citronella and thyme in killing adult female *Culex pipiens* in a glass chamber. By using "electrical" vaporizing equipment, EOs at concentrations of 1.25, 2.5, 5, 10 and 20 **mg/m^3** were able to evaporate. The number of adult female mosquitoes that were knocked down was noted after 10, 30, and 60 minutes. After

60 minutes, the citronella and thyme oils' KC_{50} values were 0.424 and 5.651 **mg/m^3**; after 30 minutes, they were 0.554 and 2.825 **mg/m^3**; and after 10 minutes, they were 11.557 and 7.198 **mg/m^3** (Table 5).

There is no knockdown impact from the deodorized kerosene (DOK) therapy. Adult *Culex pipiens* were exposed to varying concentration of thyme oil, citronella oil, and their nanoemulsions; the results were recorded after a 24-hour. According to Table 6, essential oils of citronella and thyme had a strong adulticidal effect on adult *Culex pipiens*. In terms of lethal concentrations (LC_{50}), the thyme and citronella oils and their nanoemulsions were 6.038 and 5.619 mg/mL of EOs and 11.048 and 20.245 mg/mL of their nanoemulsion, respectively.

These findings suggest that EOs are typically more active than their nanoformulations. Loss of activity may result from the evaporation of oils during the nanoformulations process or from the effects of the sonication power used throughout the process. Moreover, the complete water solubility of nanoemulsions promises their use in a range of watery environments. The efficacy of these formulations is enhanced by small droplet sizes, particularly in the nanometer range. The significantly large surface area of nanodroplets is one of their essential properties.

This characteristic results in higher penetration capabilities, wider contact areas, and rapid delivery to targeted sites of action. It is expected that these dynamics will optimize the effectiveness of citronella and thyme nanoemulsions against insects. Citronella and thyme oils may possess insecticidal properties due to active components such as beta-geraniol, e-citral, linalool, o-cymene, camphor, myrcene, and thymol. The insecticidal activity of citronella and thyme essential oils could stem from their action on one or more insect targets, including the inhibition of acetylcholine esterase (AChE) enzyme or the blocking of octopamine or GABA receptors [30,31].

This research aligns with several United Nations Sustainable Development Goals (SDGs). It supports **SDG 3 (Good Health and Well-being)** by contributing to vector control strategies aimed at reducing mosquito-borne diseases. It advances **SDG 6 (Clean Water and Sanitation)** through the promotion of environmentally friendly insecticides that reduce water pollution. Furthermore, it contributes to **SDG 12 (Responsible Consumption and Production)** by encouraging the use of natural and sustainable pest control methods, and to **SDG 15 (Life on Land)** by helping to protect terrestrial ecosystems from the adverse effects of synthetic chemical pesticides. By highlighting these connections, the study underscores its broader societal and environmental relevance and its alignment with the global 2030 Agenda for Sustainable Development [32].

3. Conclusions

In conclusion, the citronella and thyme essential oils and their nanoemulsions are water-based and solvent-free, suggesting a low toxic effect on animals and an eco-friendly profile. It also demonstrates potential insecticidal action against *Culex* pipiens. Therefore, these oils offer promises for creating novel adulticides as a substitute for synthetic pesticides.

4. Conflicts of interest

The authors declare no conflict of interest.

5. Formatting of funding sources

6. Funding and Acknowledgments

Financial support for this work was provided by the National Research Centre (NRC) (NRC Project No. 12020201).

7. Acknowledgments

The authors acknowledge that this research is derived from a Master's thesis and thank the NRC for the essential resources and support required to complete this research.

8. References and Bibliography

[1] Becker, N., Petrić, D., Boase, C., Lane, J., Zgomba, M., Dahl, C., Kaiser, A., (2003). Mosquitoes and Their Control, Mosquitoes and Their Control. Springer US.
[2] World Health Organization, (2012). Management of severe malaria: a practical handbook, 3rd ed.
[3] Ranson H, Lissenden N. Trends Parasitol. (2016) Insecticide Resistance in African Anopheles Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control. Mar; 32 (3):187-196. doi:10.1016/j.pt.2015.11.010.Epub2016Jan27.Review.
[4] Moyes, C. L., Vontas, J., Martins, A. J., Ng, L. C., Koou, S. Y., Dusfour, I., ... &Weetman, D. (2017). Contemporary status of insecticide resistance in the major Aedes vectors of arboviruses infecting humans. PLoS neglected tropical diseases, 11(7), e0005625.
[5] Mortuza, T., Chen, C., White, C. A., Cummings, B. S., Muralidhara, S., Gullick, D., & Bruckner, J. V. (2018). Toxicokinetics of deltamethrin: dosage dependency, vehicle effects, and low-dose age-equivalent dosimetry in rats. Toxicological Sciences, 162(1), 327-336.
[6] Mossa,A.H.,Refaie,A.A.,Ramadan,A.&Bouajila,J.(2013).Amelioration of prallethrin-inducedoxidativestress and hepatotoxicity in rat by the administration of Origanummajoranaessentialoil.BioMedRes.Inter.2013:1-11.

[7] Mohafrash, S.M.M., Abdel-Hamid, H.F. &Mossa, A.H. (2017). Adverse effects of sixty days sub-chronic exposure to β–cyfluthrin on male rats. Journal of Environmental Science and Technology, 10 (1):1-12.
[8] Mossa, A.T.H. (2016).Green pesticides: Essential Oils as biopesticides in insect-pest management.JournalofEnvironmentalScienceandTechnology,9 (5):354-378.
[9] Mossa, A.H., Abdelfattah, N.A. &Mohafrash S.M.M. (2017). Nanoemulsion of camphor (Eucalyptus globulus) essential oil, formulation, characterization and insecticidal activity against Wheat Weevil, Sitophilusgranarius. Asian Journal of Crop Science, 9(3): 50-62.
[10] Mossa, A.H., Afia, S.I., Mohafrash, S.M.&Abou-Awad, B. A. (2018). Formulation and characterization of garlic (Allium sativum L.) essential oil nanoemulsion and its acaricidal activity on eriophyid olive mites (Acari: Eriophyidae). Environmental Science and Pollution Research (In press).
[11] Abbas, M. G., Haris, A., Binyameen, M., Nazir, A., Mozūratis, R., & Azeem, M. (2022). Chemical composition, larvicidal and repellent activities of wild plant essential oils against Aedes aegypti. Biology, 12(1), 8.
[12] Martın, A., Varona,S.,Navarrete,A. &Cocero,M.J.(2010).Encapsulationandco-precipitationprocesses with supercrtitical fluids: applications with essential oil. The Open Chemical Engineering Journal,4:31–41.
[13] Kauffman, E., Payne, A.,Franke, M. A., Schmid, M. A., Harris, E., & Kramer, L. D. (2017). Rearing of Culex spp. and Aedes spp. mosquitoes. Bio-protocol, 7(17).
[14] Sawicki, R. M. (1964). Some general considerations on housefly rearing techniques. Bulletin of the World Health Organization, 31(4), 535.
[15] Vilasau, J., Solans, C., Gómez, M. J., Dabrio, J., Mújika-Garai, R., & Esquena, J. (2011). Phase behaviour of a mixed ionic/nonionic surfactant system used to prepare stable oil-in-water paraffin emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 384(1-3), 473-481.
[16] Mossa, A. T. H., Abdelfattah, N. A. H., &Mohafrash, S. M. M. (2019). Rosemary essential oil nanoemulsion, formulation, characterization and acaricidal activity against the two spotted spider mite Tetranychusurticae (Acari: Tetranychidae). Journal of Plant Protection Research, 59 (1): 102–112.
[17] Shafiq, S., &Shakeel, F. (2010). Stability and self-nanoemulsification efficiency of ramipril nanoemulsion containing labrasol and plurololeique. Clinical Research and Regulatory Affairs, 27(1), 7-12.
[18] Sugumar, S., Clarke, S. K., Nirmala, M. J., Tyagi, B. K., Mukherjee, A., &Chandrasekaran, N. (2014). Nanoemulsion of eucalyptus oil and its larvicidal activity against Culex quinquefasciatus. Bulletin of entomological research, 104(3), 393-402.
[19] Dey, P., Mandal, S., Goyary, D., & Verma, A. (2023). Larvicidal property and active compound profiling of Annona squamosa leaf extracts against two species of diptera, Aedes aegypti and Anopheles stephensi. Journal of Vector Borne Diseases, 60(4), 401-413.
[20] World Health Organization. (2022). Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions. World Health Organization.
[21] World Health Organization.(1998). Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces: report of the WHO informal consultation, Geneva, 28-30 September 1998 (No.WHO/CDS/CPC/MAL/98.12).
[22] Abbott W.S (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
[23] Finney D. (1971). Probit Analysis Cambridge Univ. Press, New York (1971), p. 319.
[24] Ashaq, B., Rasool, K., Habib, S., Bashir, I., Nisar, N., Mustafa, S., Ayaz, Q., Nayik, G.A., Uddin, J., Ramniwas, S. and Mugabi, R., 2024. Insights into chemistry, extraction and industrial application of lemon grass essential oil-A review of recent advances. Food Chemistry: X, p.101521.
[25] Firdausiah, S., Firdaus, F., Sulaeha, S., Rasyid, H. and Amalia, S.F., 2023. Citronella extracts: Chemical composition, in vivo and in silico insecticidal activity against fall armyworm (Spodoptera frugiperda JE Smith). Egyptian Journal of Chemistry, 66(7), pp.235-243.
[26] Harianingsih, H., Wulandari, R., Harliyanto, C. and Andiani, C.N., 2017. Identifikasi GC-MS ekstrak minyak atsiri dari sereh wangi (Cymbopogon winterianus) menggunakan pelarut metanol. Techno (Jurnal Fakultas Teknik, Universitas Muhammadiyah Purwokerto), 18(1), pp.23-27.
[27] Salem, M.A., De Souza, L.P., Serag, A., Fernie, A.R., Farag, M.A., Ezzat, S.M. and Alseekh, S., 2020. Metabolomics in the context of plant natural products research: from sample preparation to metabolite analysis. Metabolites 10: 37. DOI: 10.3390/metabo10010037.
[28] Mossa, A.T.H., Mohamed, R.I. and Mohafrash, S.M., 2022. Development of a ‘green’nanoformulation of neem oil-based nanoemulsion for controlling mosquitoes in the sustainable ecosystem. Biocatalysis and Agricultural Biotechnology, 46, p.102541.
[29] Almeida, F., Correa, M., Zaera, A.M., Garrigues, T. and Isaac, V., 2022. Influence of different surfactants on development of nanoemulsion containing fixed oil from an Amazon palm species. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 643, p.128721.
[30] Mohafrash, S. M., Fallatah, S. A., Farag, S. M. & Mossa, A. T. H. (2020). Mentha spicata essential oil nanoformulation and its larvicidal application against Culex pipiens and Musca domestica. Industrial Crops and Products, 157: 112944.
[31] Magierowicz, K., Gorska-Drabik, E. & Golan, K. (2020). Effects of plant extracts and essential oils on the behavior of Acrobasis advenella (Zinck.) caterpillars and females. Journal of Plant Diseases and Protection, 127(1): 63-71.
UN, G. A. (2015). Transforming our world: The 2030 agenda for sustainable development. Division for Sustainable Development Goals. New York: World Health Organization.