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Green and Effective Plant Essential oil Nanoemulsion for the German cockroach Control: A Novel and Environmentally Friendly Approach

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ABSTRACT

The German cockroach *Blattella germanica* L. poses a serious threat to human health and environmental hygiene. Plant essential oils are considered suitable alternatives to chemical insecticides for controlling these common household pests. This study aimed to develop nanoemulsions of *Rosmarinus officinalis* L. (rosemary) and *Eucalyptus globulus* L. (eucalyptus) essential oils and evaluate their insecticidal activity against the German cockroach. Essential oils were extracted using a Clevenger apparatus. Nanoemulsions of rosemary, eucalyptus, and their 1:1 combination were prepared and characterized by particle size analysis (PSA) and transmission electron microscopy (TEM). Bioassays were conducted using the standard contact method with a 30-minute exposure at concentrations of 5%, 7.1%, 10%, 14.1%, and 20%, with acetone as the control, on male German cockroaches under laboratory conditions. Mortality was recorded after 24 hours. The average particle sizes were 84.46 nm for eucalyptus, 91.33 nm for rosemary, and 99.16 nm for the combined nanoemulsion, all exhibiting spherical morphology. The lethal concentration values (LC_{50} and LC_{95}) were 4.08% and 10.33% for eucalyptus, 4.79% and 11.31% for rosemary, and 7.09% and 26.56% for the combined nanoemulsion, respectively. The results indicate that individual nanoemulsions have higher efficacy compared to their combination, highlighting the importance of assessing both separate and combined effects to fully understand their insecticidal potential. These findings emphasize the potential of essential oil nanoemulsions as eco-friendly alternatives for managing German cockroach infestations.

Keywords: *Blatella germanica*, *Rosmarinus officinalis*, *Eucalyptus globulus*, Essential oil

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INTRODUCTION

The German cockroach, *Blattella germanica*, is a pervasive urban pest known for its role in transmitting pathogens, triggering allergic reactions, and contaminating food sources, thereby posing significant public health risks worldwide [1]. Traditionally, chemical insecticides such as pyrethroids, organophosphates, and carbamates have been the primary tools for controlling cockroach populations. However, the widespread and often indiscriminate use of these synthetic insecticides has led to the rapid development of resistance in German cockroach populations, severely undermining their effectiveness [2,3]. For example, resistance ratios exceeding 4000-fold to cypermethrin and significant resistance to fipronil and chlorpyrifos have been documented, complicating pest management efforts [4]. Moreover, the environmental and health concerns associated with chemical insecticides, including toxicity to non-target organisms and potential human exposure risks, have intensified the demand for safer, sustainable alternatives [5].

In recent years, plant-derived essential oils (EOs) have gained considerable attention as eco-friendly insecticidal agents due to their biodegradability, low mammalian toxicity, and diverse modes of action against insect pests [6,7]. Numerous studies have demonstrated the potent insecticidal and repellent properties of EOs extracted from plants such as *Eucalyptus camaldulensis*, *Artemisia sieberi*, *Piper nigrum*, and *Nepeta cataria*, and *Francoeuria undulata* against the German cockroach [8-11]. For instance, essential oil from *F. undulata* has been reported to cause 100% mortality within 24 hours at specific concentrations [10]. Thyme, sweet orange, and lavender oils achieved knockdown in less than 30 s and 100% mortality in 24h [11]. Despite their promising bioactivity, the practical application of EOs is often limited by their high volatility, poor water solubility, and susceptibility to environmental degradation, which can reduce their efficacy and residual activity [12,13]. To overcome these limitations, nanoemulsion technology has emerged as a cutting-edge strategy to enhance stability, bioavailability, and controlled release of essential oils. Nanoemulsions are fine oil-in-water dispersions with droplet sizes typically below 200 nm, which improve the solubility and penetration of hydrophobic compounds like EOs, leading to increased insecticidal potency and prolonged action [14]. Additionally, nanoemulsions are generally prepared using biodegradable and non-toxic surfactants, aligning with the principles of green chemistry and sustainable pest management

[15]. Recent studies have demonstrated that EO-based nanoemulsions exhibit enhanced insecticidal and repellent activities against various insect pests, including mosquitoes and agricultural pests, but their application against urban pests like the German cockroach remains underexplored [14-15]. Iran’s diverse ecological habitats provide an ideal environment for cultivating a wide range of plant species, offering cost-effective and readily accessible resources for the development of botanical pesticides [16]. Among them, *Rosmarinus officinalis* L., commonly known as rosemary, is a perennial herb belonging to the Lamiaceae family. Native to the western Mediterranean region, rosemary typically grows as a small evergreen shrub and is extensively utilized in the pharmaceutical, cosmetic, and hygiene industries due to its valuable essential oil. Rosemary essential oil has been reported to have an insecticidal effect against the German cockroach. This study show that its toxicity increases with concentration and exposure time, resulting in significant mortality in cockroaches. Specifically, it has demonstrated strong contact toxicity and fumigant effects and has been considered one of the most effective essential oils for controlling German cockroaches in laboratory tests [17]. Similarly, *Eucalyptus globulus* L., a member of the Myrtaceae family comprising over 800 species, is native to Australia but has been widely introduced and cultivated in tropical and subtropical regions worldwide. This fast-growing and adaptable species is prized not only for its timber, gum, and cellulose but also for its essential oils, which have been employed in traditional medicine for centuries. The essential oils extracted from eucalyptus leaves and other aerial parts exhibit diverse bioactive properties, making them important candidates for natural pest management applications including cockroaches [18]. Toxicity evaluations demonstrated significant bioactivity of these essential oils, both individually and in combination, against *B. germanica* confirming their potential as effective natural insecticides. The study also highlighted variations in chemical profiles depending on extraction and environmental factors, which may influence their bioefficacy [17-19].

By integrating natural bioactive compounds with advanced nanotechnology, this research aims to advance sustainable urban pest management strategies that reduce environmental impact while ensuring high efficacy in pest control. Therefore, the objective of this study was to develop nanoemulsions of eucalyptus and rosemary essential oils, both individually and in combination, and to evaluate their insecticidal activity against the German cockroach (*B. germanica*), one of the most prevalent urban pests, under laboratory conditions.

MATERIALS AND METHODS

German cockroach

A susceptible strain of German cockroaches was maintained in the insectarium of the Department of Vector Biology and Control of Diseases at Ahvaz Jundishapur University of Medical Sciences. The cockroaches were fed a diet consisting of toast, biscuits, bread, and mouse food. The rearing containers were kept under controlled conditions of 27 ± 2 °C temperature, 55 ± 5% relative humidity, and a 12:12 hour L: D photoperiod [19].

Preparation of Plant EOs

Fresh leaves of *Eucalyptus globulus* (Herbarium Code: A241850201LP) and *Rosmarinus officinalis* (Herbarium Code: A241600201LP) were collected from the medicinal plant garden at the School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran), and air-dried in the shade at room temperature to preserve their volatile compounds. The dried leaves were then ground into a fine powder using an electric grinder. Essential oils were extracted from 100–130 g of the powdered leaves by hydrodistillation using a Clevenger apparatus with distilled water as the extraction medium. The distillation process was carried out for 3 hours, after which the essential oils were collected, separated from the aqueous phase, and stored in amber vials at 4 °C until further analysis.

Plant Analysis by Gas-chromatography Mass Spectrometer (GC-MS)

GC-MS was used for EOs analysis. It is equipped with HP–5MS column (30 m× 0.25 mm× 0.25 µm). The initial temperature used was 40 °C for 1 min and was later raised to 220 °C at a rate of 3 °C/ min and finally raised to 270 °C for 5 min at a rate of 20 °C/min. Other parameters of the GC-MS included carrier gas Helium (99/999 %), injector temperature (260 °C), detector temperature (FID, 270 °C), split-less mode, ionization potential of 70eV, scan rate of 1 scan/sec, the scan range of 40–48 m/z was used for all analysis. The essential oil components were identified by comparing their retention indices mass spectra fragmentation with those in a stored Wiley 7n.1 mass computer library and those of the National Institute of Standards and Technology (NIST) [19].

Preparation of the EOs Nanoemulsion

Nanoemulsions containing 20% (v/v) essential oils of eucalyptus, rosemary, and their 1:1 combination were prepared using a low-energy emulsification method. The aqueous phase was prepared by mixing 68 mL of distilled water with 5 mL of Tween 80 surfactant and homogenized at 6000 rpm for 5 minutes. The oil phase consisted of 20 mL of essential oils, 5 mL of castor oil as a carrier, and 2 g of lecithin as a natural emulsifier, which were thoroughly mixed to homogeneity for 3 minutes. The oil phase was added dropwise to the cooled aqueous phase (0 °C) under continuous homogenization at 6000–7000 rpm. Homogenization continued for 30 minutes to ensure stable nanoemulsion formation. All nanoemulsion formulations were visually examined after 24 hours and during 15 days to ensure physical stability without phase separation (Fig. 1) [20].

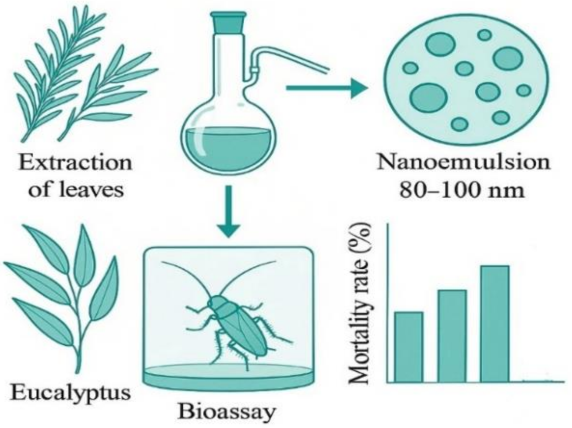

Fig. 1 Graphic summary of the development and evaluation of essential oil nanoemulsion against the German cockroach.

Characterization of EOs Nanoemulsion

Particle Size Analyzer (PSA) was used to determine the average size of the prepared nanoparticles with Dynamic Light Scattering (DLS) method in a wavelength of 633 nm in three repetitions in school of Pharmacy, Ahvaz Jundishapur University of Medical Sciences. The morphology of the freeze-dried nanoparticles of the formulations was investigated by transmission electron microscopy (TEM Model 906 E, LEO Company) in Central Laboratory, Shahid Chamran University (Ahvaz, Iran).

Bioassay Evaluation against *B. germanica*

The susceptibility of *B. germanica* to nanoemulsions of eucalyptus, rosemary, and their 1:1 combination was assessed following the standard WHO bioassay method. The concentrations of 5%, 7.1%, 10%, 14.1%, and 20% were selected based on a logarithmic scale derived from preliminary bioassays to accurately determine the dose-response relationship. Nanoemulsion solutions were prepared using acetone as the solvent. For each concentration, 2 mL of the nanoemulsion was poured into a 600 mL glass beaker, which was then rolled horizontally to ensure uniform coating of the inner surface. After the solvent evaporated and the beakers were completely dry, ten adult male or late-instar nymph cockroaches were introduced into each container and exposed to the treated surfaces for 30 minutes. Subsequently, the insects were transferred to clean containers and maintained under controlled conditions at 27 ± 2 °C, 55 ± 5% relative humidity, and a 14:10 h light-dark photoperiod. A control group was exposed to acetone only. Mortality was recorded after 24 hours. Each treatment was replicated three times to ensure statistical reliability [21].

Data Analyzing

The mortality data were first corrected using Abbott's formula, considering the mortality in the control group, and then analyzed using SPSS 26 software. To determine the concentration required to kill 50% and 95% of the cockroach at different concentrations, the probit regression model was used. To compare the mean mortality data, if the data distribution was normal and variances were equal, ANOVA and LSD test were applied at $\alpha = 0.05$; otherwise, the Kruskal-Wallis test were used.

RESULTS

Plant EOs Components

A total of 36 components were identified in rosemary essential oil, with 26 compounds, comprising 92.6% of the total, detailed in Table 1. The major constituents included α-pinene (20.67%), verbenone (11.8%), camphor (10.69%), 1, 8-cineole (9.35%), borneol (9.06%), and camphene (7.15%). Among these, 1, 8-cineole, camphor, borneol, and α- pinene are well-recognized for their insecticidal and repellent activities against various insect pests. These oxygenated monoterpenes and monoterpene hydrocarbons contribute significantly to the bioactivity of rosemary oil, making it a promising natural agent for pest management applications (Table 1).

In the essential oil of eucalyptus, a total of 27 components were identified with 18 compounds, comprising 91.02% of the total, detailed in Table 1. The oil was predominantly composed of 1, 8-cineole (50.67%), followed by α- pinene (17.48%), aromadendrene (4.27%), and limonene (4.26%). These major constituents represent a mixture of monoterpenes and sesquiterpenes known for their characteristic aroma and bioactive properties. The high concentration of 1, 8- cineole, a well-known oxygenated monoterpene, largely defines the typical eucalyptus scent and contributes to the essential oil's pharmacological effects. α- Pinene and limonene, both monoterpene hydrocarbons, along with sesquiterpenes such as aromadendrene and azulene, add complexity to the chemical profile, potentially enhancing the oil's therapeutic potential (Table 1).

Table 1 GC-MS analysis components of *Rosmarinus officinalis* and *Eucalyptus globulus* essential oils.

	R. officinalis				*Eu. globulus*		
Components	Major Constituents (%)	RI	RT	Components	Major Constituents (%)	RI	RT
Tricyclene	0.46	919	7.447	α-Pinene	17.48	930	7.819
α- Pinene	20.67	930	7.825	β-Pinene	0.79	971	9.015
Camphene	7.15	943	8.208	β-Myrcene	1.05	987	9.461
Verbenene	0.68	959	8.363	1-Phellandrene	0.2	1001	9.833
β- Pinene	0.71	971	9.003	Limonene	4.26	1021	10.565
Octanone	2.39	985	9.318	1,8-Cineole	50.67	1024	10.731
β-Myrcene	2.45	987	9.456	γ-Terpinene	0.68	1051	11.452
Octanol	0.2	988	9.581	α-Terpinolene	2.48	1084	12.328
α-Terpinene	0.32	1011	10.182	trans-Pinocarveol	0.19	1132	13.804
p- Cymene	1.82	1017	10.423	Terpinen-4-ol	1.26	1170	14.92
Limonene	4.88	1021	10.554	α-Terpinoel	1.99	1184	15.309
1,8- Cineole	9.35	1024	10.634	Isoledene	0.17	1370	20.31
γ- Terpinene	0.26	1051	11.435	α-Gurjunene	1.29	1406	21.265
α- Terpinolene	0.6	1084	12.316	Gurjunene <β->	0.2	1427	21.838
Linalool	1.98	1091	12.666	Aromadendrene	5.93	1435	22.015
filifolone	0.52	1101	12.763	Ledene	1.98	1493	23.394
Camphor	10.69	1139	13.987	Amorphene <δ->	0.15	1508	23.84
Pinocarvone	0.14	1157	14.491	δ-Cadinene	0.25	1518	24.046
Borneol	9.02	1163	14.622				
Pinocamphone	0.93	1169	14.834				
Terpineol	1.22	1184	14.92				
Verbenone	11.8	1201	15.893				
Bornyl acetate	3	1280	17.97				
Piperitenone	0.23	1337	19.44				
Trans Caryophyllene	0.95	1416	21.512				
α- Humulene	0.18	1450	22.364				

RT: Retention time, RI: Retention Index

TEM and PSA Results of EOs nanoemulsions

Table 2 shows particle sizes (nm) of nanoemulsions of eucalyptus (100-150 nm), rosemary (50-100nm) EOs and their 1:1 mixture (100-150 nm) measured by PSA (dynamic light scattering) at 20% concentration. The averages of three NPs size (mean ± SD) were measured as 95.8 ± 8.5 nm, 91.3 ± 6.4 nm, and 99.2 ± 10.9 nm, respectively. TEM photographs revealed that the nanoparticles have a spherical morphology and the particle size is in good agreement with the dynamic light scattering analysis (Fig. 2). The corresponding DLS graphs display a sharp peak in all three nanoformulations, it typically signifies a uniform and narrow particle size distribution.

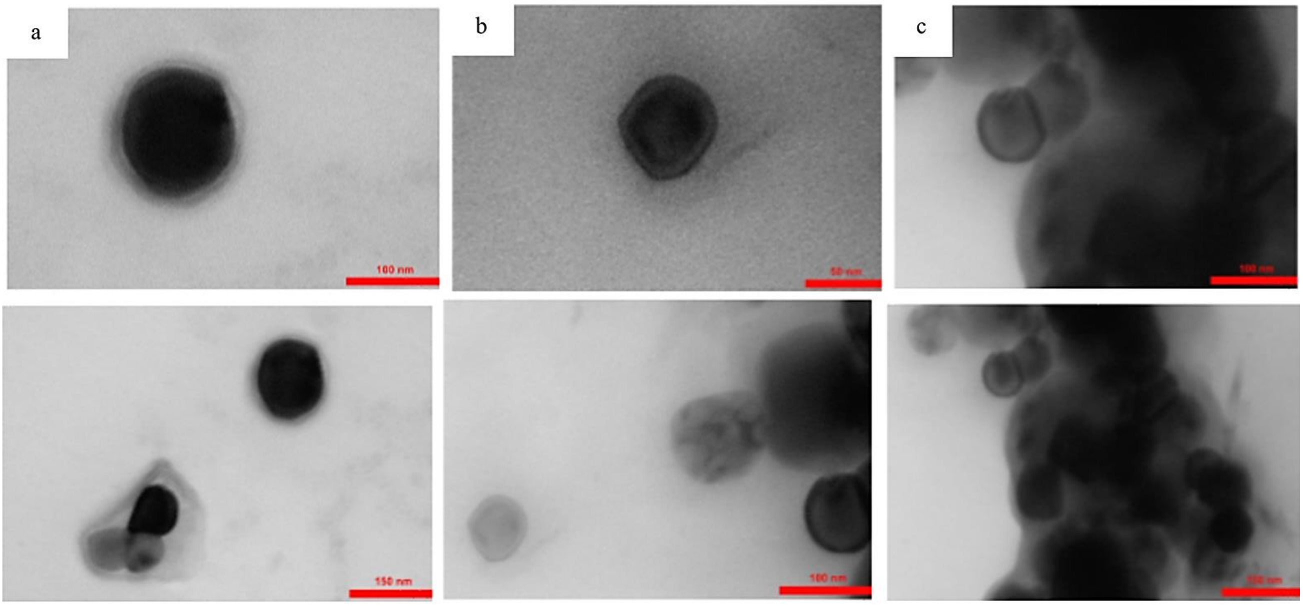

Fig. 2 TEM images of the nanoparticles (a) eucalyptus (b) rosemary (c) eucalyptus+ rosemary mixture

Table 2 Average particle size of eucalyptus, rosemary, and their combination (1:1) nanoemulsions determined by Particle Size Analysis (PSA)

Nanoemulsion	Analysis 1 (nm)	Analysis 2 (nm)	Analysis 3 (nm)	NP_S size ± SD (nm)
Eucalyptus	128	92.3	83.1	95.8 ± 8.5
Rosemary	86.8	104	83.2	91.3 ± 6.4
Rosemary + Eucalyptus	77.5	112	108	99.2 ± 10.9

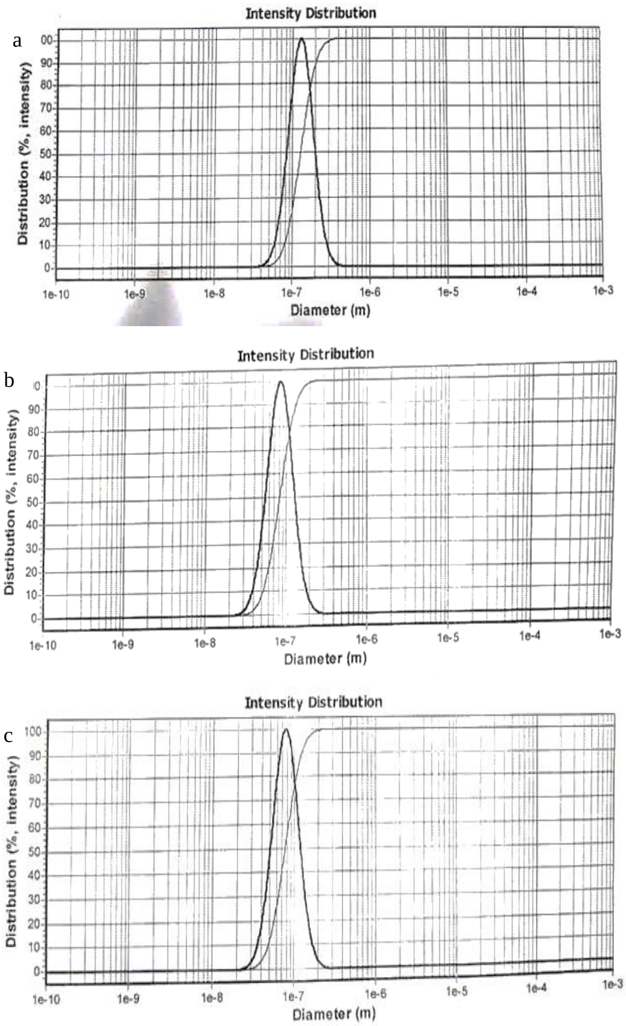

Fig. 3 Dynamic light scattering (DLS) graphs of (a) eucalyptus, (b) rosemary, and (c) mixture nanoemulsions.

This means many particles in the sample are of very similar size, indicating low size dispersion. Such a characteristic point to the sample being monodisperse, which is highly desirable for nanoemulsions and nanoparticles. Consequently, a sharp peak often correlates with high nanoemulsion stability, as the particles have not aggregated or agglomerated and their sizes remain consistent, reducing the likelihood of sedimentation or phase separation. Furthermore, a sharp DLS peak is usually accompanied by a low polydispersity index (PDI)**,** typically below <1, further confirming the narrow and homogeneous particle size distribution (Fig. 3).

Mortality Rates and Lethal Concentrations of EOs Nanoemulsions against Cockroach

The insecticidal activity of rosemary and eucalyptus nanoemulsions against *B. germanica* exhibited a clear concentration-dependent response. Mortality rates for rosemary ranged from 60% to 100%, while those for eucalyptus ranged from 50% to 100%. Both individual nanoemulsion achieved 100% mortality at concentrations of 14.1% and 20%. At lower concentrations, rosemary showed slightly higher mortality compared to eucalyptus. Statistical analysis revealed a significant difference in mortality rates across concentrations for the rosemary-eucalyptus mixture ($P = 0.0394$), whereas no significant difference was found between the individual nanoemulsions ($P = 0.4654$) (Fig. 4). Probit analysis supported these findings, with overlapping confidence intervals for the LC_{50} and LC_{95} values of the individual nanoemulsions, indicating comparable potency. In contrast, the combined rosemary-eucalyptus nanoemulsion demonstrated notably reduced efficacy, with mortality ranging from 30% to 94% and substantially higher lethal concentrations (LC_{50} and LC_{95}), suggesting a likely antagonistic interaction between the two oils. The heterogeneity factors for all treatments were below 1, indicating a good fit of the bioassay data to the Probit model (Table 3). Overall, although all formulations were capable of reaching high mortality, the individual EO nanoemulsions were more effective and efficient insecticidal agents against *B. germanica* compared to their mixture, highlighting a potential antagonistic effect upon combination.

Comparison of Toxicity of Nanoemulsion and Unformulated EOs

The comparison was made at concentrations of 5%, 7.1%, 10%, 14.1%, and 20%. The LC_{50} values for unformulated and nanoemulsion EOs are shown in Figure 4. The highest value belongs to the unformulated EO of eucalyptus with LC_{50} = 10.24%, while the lowest value belongs to the rosemary + eucalyptus mixture with LC_{50} = 3.17% [19]. Although expressing the EOs as nanoemulsion significantly reduced the LC_{50} values of the individual oils, the lethal concentration increased in the combined nanoemulsion, leading to a decrease in insecticidal potency. The non-formulated mixture of rosemary + eucalyptus EOs exhibited the highest lethality with the lowest LC_{50} value of 3.17% among all treatments (Fig. 5).

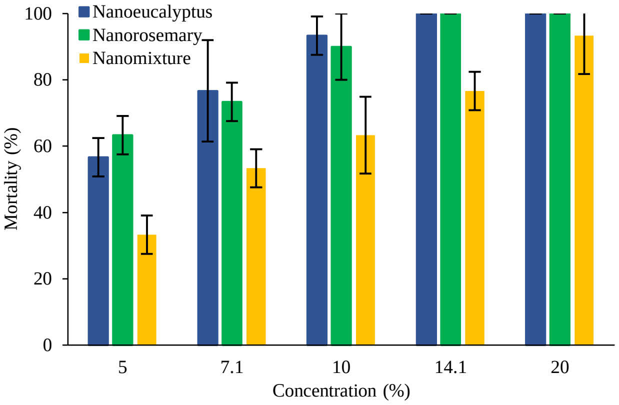

Fig. 4 Mean mortality rates of the German cockroach exposed to nanoemulsion EOs of *R. officinalis* and *Eu. globulus* individually and in mixture using the contact bioassay.

Table 3 Lethal Concentrations of rosemary, eucalyptus nanoemulsion and their mixture against *B. germanica*

Nanoemulsion	LC_{50} (Cl)	LC_{95} (Cl) *	Slop (± SE)	ℵ(df)
eucalyptus	4.79 (3.47 – 5.61)	10.33 (8.65 – 15.12)	4.9 (± 0.92)	5.12 (13)
rosemary	4.08 (3.26 – 5.77)	11.31 (9.25 – 17.68)	4.08 (± 0.82)	5.53 (13)
rosemary + eucalyptus	7.09 (5.43 – 8.49)	26.56 (19.03 – 53.85)	2.87 (± 0.56)	6.07 (13)

*CI (Confidence Interval), Heterogeneity Factor (hf) = $\frac{\aleph}{df}$, when the heterogeneity factor is >1, a plot of the data should be examined because the data do not fit the model.

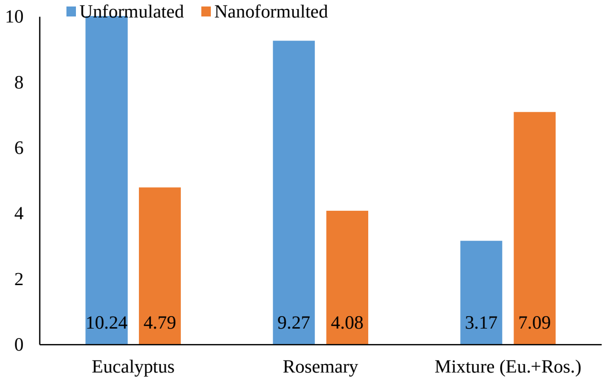

Fig. 5 Lethal concentrations of unformulated and nanoemulsified EOs of eucalyptus, rosemary and their mixture against the German cockroach under laboratory condition

DISCUSSION

The increasing interest in nanoemulsions as advanced delivery systems for essential oils (EOs**)** stems from their ability to address key limitations of conventional EOs, namely volatility, lipophilicity, limited aqueous solubility, and instability. These physicochemical drawbacks notably restrict the practical use of EOs in pest control, where sustained efficacy and controlled release are essential for field application. By enhancing water miscibility, dispersibility, and bioavailability, nanoemulsions offer marked improvements over unformulated EOs, particularly in improving persistence and insecticidal activity [22].

Our study contributes by developing and characterizing nanoemulsions of *Eu. globulus* and *R. officinalis* oils, then evaluating their toxicity against the German cockroach (*B. germanica*), an important urban pest. Eucalyptus and rosemary EOs are known to be rich sources of bioactive terpenes such as 1,8-cineole, α- pinene, limonene, camphor, borneol, and camphene, whose insecticidal and repellent activities have been extensively documented, for instance, 1,8- cineole acts as a neurotoxin disrupting insect neural signaling, while α- pinene primarily serves as a repellent [17, 19, 23, 24]. Synergistic interactions among these compounds may target multiple physiological pathways in pests, enhancing overall insecticidal efficacy [25].

Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) characterization confirmed that our nanoemulsions had spherical particles ranging from approximately 50 to 150 nm, consistent with previous reports on EO nanoemulsions [23, 24]. Similar approaches in preparing rosemary and eucalyptus EO nanoemulsions yielded particle sizes within this range, with surfactant composition and sonication parameters critically influencing morphology and stability [22, 23]. Further, previous studies evaluating nanoencapsulated chamomile EO against *Periplaneta americana* reported size reductions from over 3μ in unformulated oil to ~220 nm in nanoemulsions [26]. The results obtained in the present study are consistent with these reports, demonstrating successful preparation of rosemary nanoemulsions with stable spherical particles, highlighting the effectiveness of the methods used and their potential application for optimized delivery of plant essential oils.

The bioassay results demonstrated significantly improved insecticidal activity of nanoemulsified *Eucalyptus* and *Rosmarinus* oils compared to their non- forms. Specifically, the LC_{50} values decreased from 10.54% and 9.27% in unformulated oils to 4.79% and 4.07% in nanoemulsions, respectively. This enhancement aligns with literature reports that nanoformulation improves efficacy by promoting better dispersion, increasing surface area for interaction, and enabling controlled release of active components, which counteract the rapid degradation typically encountered with EOs [21, 22, 27]. Comparable studies have shown that polymeric nanoparticles of peppermint and palmarosa EOs enhanced insecticidal activity against *B. germanica* up to 8- to 10-fold, indicated by significant reductions in LC_{50} values. Nanoparticles of peppermint and palmarosa essential oils improved the effectiveness of these oils by preventing rapid evaporation, increasing contact surface area, and enhancing absorption and interaction with biological tissues. The LC_{50} values of peppermint and palmarosa nanoemulsions against the German cockroach were reported as 31.43 μg/cm² and 25.41 μg/cm², respectively, which demonstrate stronger insecticidal activity compared to their non-nano counterparts, which had LC_{50} values of approximately 245.95 μg/cm² and 246 μg/cm², respectively. Likewise, geranium and bergamot EO nanoemulsions exhibited nearly doubled toxicity compared to original oils, facilitating reduced recommended application doses and potentially decreasing environmental impact [27]. Similarly, the toxicity of nanoparticles of geranium and bergamot essential oils against the German cockroach was evaluated by comparing lethal concentration 50 (LC_{50}) values. For non-formulated essential oils, the LC_{50} values were 298.3 μg/cm² for geranium and 947.4 μg/cm² for bergamot. After nano-formulation, the LC_{50} decreased significantly to 139.1 μg/cm² for geranium and 591.8 μg/cm² for bergamot, indicating that the nano-formulation enhanced the toxicity of both oils. Geranium essential oil showed higher toxicity than bergamot in both formulations. Additionally, nanoparticles increased the repellent effect and negatively affected the nutritional parameters of the cockroach [21].

Our findings support and extend these observations, emphasizing that nanoemulsified eucalyptus and rosemary oils are promising, eco-friendly alternatives for cockroach control. Interestingly, the nanoemulsion of the *eucalyptus + rosemary* mixture showed less insecticidal activity (LC_{50} = 7.09%) compared to the non-formulated mixture (LC_{50} = 3.23%). In multicomponent nanoemulsions, physicochemical interactions among the various bioactive compounds and emulsifiers often lead to the formation of complex structures that can restrict or slow the release of the active ingredients. The presence of multiple bioactive components with differing properties may reduce the overall stability of the emulsion particles and cause antagonistic effects, such as competitive adsorption at interfaces or functional interference among components. These phenomena generally result in decreased release rates and lower bioavailability of the active substances at the target site, ultimately diminishing the toxicity or efficacy of the formulation [28, 29]. Such findings underscore the importance of optimizing blend ratios, emulsifier types, and process parameters for multi-oil nanoformulations to preserve or enhance synergistic effects.

Nanoformulations also improve stability, a critical factor due to the volatility and degradation of EOs under environmental stressors like heat, light, and oxygen exposure [22, 25]. Our nanoemulsions remained physically stable for six months without phase separation, indicating improved shelf life. However, we acknowledge the limitation that quantitative stability assays—such as repeated zeta potential measurements and accelerated aging tests—were not conducted, which are essential for comprehensive stability evaluation.

This study has some limitations. First, while physical stability was observed, detailed quantitative assessments—such as zeta potential, polydispersity index over extended periods, and environmental stress testing—were not performed, limiting stability characterization. Second, the decreased efficacy seen in the nanoemulsified EO mixture requires dedicated studies on molecular interactions and formulation optimization to prevent antagonistic effects. Third, insecticidal assays were performed in controlled laboratory conditions, necessitating field trials to examine efficacy under environmental variables. Finally, non-target organism toxicity and ecological safety assessments were not included but are crucial for evaluating environmental compatibility.

CONCLUSION

Our findings provide significant evidence that nanoemulsification of *Eu. globulus* and *R. officinalis* essential oils markedly enhances their insecticidal potency against *B. germanica* by improving solubility, stability, and controlled release of bioactive components. While individually nanoemulsified EOs achieved better toxicity profiles than unformulated oils, mixed EO nanoemulsions require careful formulation to avoid loss of synergistic efficacy. This research supports the advancement of nanoemulsified plant-derived EOs as sustainable, effective, and environmentally friendly alternatives to synthetic pesticides in integrated pest management. Future work should prioritize comprehensive stability analysis, molecular-level interaction studies in blends, expanded bioassays including field evaluations, and environmental risk assessments to fully realize the potential of these nanoformulated biopesticides.

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Authorship Contribution Statement

Mona Sharififard: Writing – original draft, Validation, Project administration, Methodology, Investigation. Marzieh Asgari-Gandoman: Data collection, writing – original draft, Moloud Kazemi: Assisted and supervised the preparation of the nanoemulsions, reviewed and approved the manuscript. Mohammad Mahmoudi Sourastani: Identified the plants, extracted essential oils, analyzed phytochemical compounds, reviewed and approved the final version of manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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