Journal of Advances in Science and Technology
Vol. 22, Issue No. 2, April-2025, ISSN 2230-9659

DOI: https://doi.org/10.29070/e000rh58

Formulation and Evaluation of Herbal Nanoemulsion for Mosquitocidal Activity Against Dengue Vector Aedes aegypti

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Abstract: The increasing resistance of Aedes aegypti to conventional insecticides has necessitated alternative vector control strategies. This study investigates the formulation and efficacy of a herbal nanoemulsion containing essential oils with mosquitocidal properties. The nanoemulsion was prepared using an ultrasonic emulsification method and characterized for particle size, zeta potential, and stability. Larvicidal and adulticidal bioassays were conducted to evaluate its effectiveness against Aedes aegypti. Results indicated significant mortality rates at low concentrations, demonstrating the potential of herbal nanoemulsions as an eco-friendly and sustainable mosquito control strategy. This study provides insights into the development of natural, nano-based solutions for dengue vector management.

Keywords: Herbal nanoemulsion, mosquito control, Aedes aegypti, dengue vector, natural insecticides, larvicidal activity

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INTRODUCTION

The mosquito is one of the most dangerous creatures that feed on blood and cause a nuisance. These parasites feed on human beings and other species for blood, and they spread illness from one person to another via their bites. A number of terrible illnesses, including dengue fever, malaria, filarial, chikungunya, japanese encephalitis, yellow fever, and others, are transmitted by mosquitoes, which are the most common types of vectors. There have been billions of deaths globally each year due to illnesses that are transmitted by mosquitoes. Aedes aegypti is a mosquito that bites during the day and is extremely anthropophilic.

The female *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) is the causative agent and primary vector for spreading Dengue and Chickungunya to the human while biting them for blood meal. The virus enters into human body during the mosquito-bite and spreads the infection by multiplication. The viral multiplication and reproduction cycles will start soon after its ingestion into the host. More than 50 million people are affected by these diseases every year (Chamberlain and Sudia, 1961; Sardar et al., 2015; Murugan et al., 2015).

In India, the dengue virus began to expand into the northern regions of the country during the years 1967 and 1968 (Ghosh et al., 1968; Myers et al., 1970). The four distinct serotypes of dengue were first reported as having been identified in South India (Rao et al., 2018). South India was the location where they were discovered. It was stated that a significant epidemic of dengue was occurring in Delhi in the year 1996. Notable instances included 10, 252 people who were discovered to be infected by this illness, and 423 people who passed away as a result of this viral infection (Balaya et al., 1967). The first documented outbreak of clinical dengue fever occurred in 1780 in Madras, now known as Chennai. Dengue fever (DF) initially surfaced on India's East Coast and in the city of Calcutta (now Kolkata) during 1963–1964 (Gupta et al., 2012).

Furthermore, according to the World Health Organization (WHO), food-borne bacterial illnesses have become a significant concern during the last few decades, affecting thousands of human communities all over the world (2020). In addition, bacterial infections are linked to the process of wound healing, which may occasionally lead to a delay in the healing process as well as a variety of other significant problems (Sabine et al., 2007; Michael et al., 1998). As a result of this, the bacterial species known as Staphylococcus aureus (Rosenbach) (Bacillales: Staphylococcaceae) poses a significant risk to human beings.

MATERIALS

Experimental animal: *Aedes aegypti* (Linnaeus, 1762)

The yellow fever mosquito is a common mosquito species that may be found worldwide. Dengue, yellow fever, chikungunya, and other diseases may be transmitted by this ectoparasite.

The peridomestic habitat of Aedes aegypti consists mostly of fresh water and includes places like tanks, pots, coolers, and other similar structures.

Three phases of Aedes aegypti's life cycle take place in water, while the fourth takes place in the air.

Essential oils: To extract the essential oils from different parts of plants, steam distillation is a common technique. Because of their hydrophobic nature and other remarkable properties, they find utility in many different circumstances. The following are some of the important differences between the four plants we studied: C. camphora, E. globulus, C. citratus, and B. campestris:

Terpene Nanoemulsions Preparation

Low-energy techniques were used to create myrcene (Myr-NE) and cyanene (Cym-NE) nanoemulsions. Using a magnetic stirrer, a 5% weight-to-weight combination of surfactants (Span 80/Tween 20) and terpene (cymene or myrcene) was introduced to the oil phase. The ultrapure water aqueous phase was added dropwise while keeping the weight-to-weight ratio at 90% after homogenisation. Sigma-Aldrich, a commercial source, provided the terpenes.

rHLB Determination of the terpenes

The rHLB values of myrcene and cymene were obtained using mixes of a hydrophilic surfactant (polysorbate 20, Tween 20; HLB 16.7) and a lipophilic surfactant (sorbitan monooleate, Span 80; HLB 4.3). The formulation with the maximum colloidal stability was determined to have the optimum rHLB value within the tested HLB range of 10.0–16.7.

Nanoemulsions Characterization

The final products were allowed to cool to room temperature before being visually assessed one hour, seven, fourteen, and twenty-one days after preparation. In addition to checking for instability symptoms like creaming, sedimentation, and phase separation, we looked at physical properties including colour, transparency, and fluidity. A 300-mesh lacy carbon film–coated copper grid was treated with nanoemulsions, and the MET Talos Arctica G2 system was used for acquisition.

Researchers used modified Franz cells to perform in vitro release investigations in a controlled environment. According to Allegra et al. and Marena et al., while Galleria mellonella larvae were used in the experiment, a number of adjustments were performed. Ten larvae (n = 10) were kept in each group at 25°C until they reached a weight of 0.2 mg; moreover, the experimental conditions required that the larvae weigh between 0.2 and 0.3 mg.

RESULTS AND DISCUSSION

The Nanoemulsions: Making and studying them

The formulation containing HLB 14 had a droplet size of 116 ± 0.40 nm on the first day (D1). Little change was observed in particle size, polydispersity index (PdI), or zeta potential levels after 21 days. Particle size, PdI, and zeta potential were all reduced in the HLB 15 formulation compared to the HLB 14 formulation. These characteristics also remained constant throughout the course of the 60-day study (Table 1). Given this, the HLB 15 formulation was chosen for the cymene rHLB.

Table1: Cym-NEs.[a]: Hydrodynamic diameter, PdI, and zeta potential

	Time	D1	D7	D14	D21	D30	D45	D60
HLB 14	**Size (nm)**	116.0 ± 0.40	111.2 ± 1.58	107.6 ± 1.59	106.5 ± 0.73	—	—	—
	PdI	0.322 ± 0.024	0.285 ± 0.007	0.331 ± 0.023	0.350 ± 0.003	—	—	—
	Zeta potential (mV)	−34.7 ± 1.1	−36.1 ± 0.7	−26.8 ± 0.4	−34.5 ± 0.8	—	—	—
HLB 15	**Size (nm)**	98.46 ± 0.83	96.74 ± 1.00	95.43 ± 1.20	98.7 ± 1.508	96.09 ± 0.61	97.69 ± 0.20	89.70 ± 0.17
	PdI	0.209 ± 0.002	0.226 ± 0.006	0.204 ± 0.006	0.216 ± 0.004	0.218 ± 0.009	0.205 ± 0.013	0.240 ± 0.004

	Zeta potential (mV)	−25.9 ± 0.43	−24.3 ± 0.80	−25.4 ± 1.45	−25.5 ± 0.68	−23.3 ± 0.45	−25.5 ± 1.14	−25.9 ± 0.35

*n = 3, mean ± standard deviation, .

Transfer electron microscopy in a cryogenic environment

Cryogenic transmission electron microscopy (cryo-TEM) is an effective approach for examining nanoemulsions (NEs) because it can highlight the complicated internal structure of colloidal systems in their natural condition. The existence of spherical droplets smaller than 180 nm was verified by the cryo-TEM data. This procedure is widely used to characterise the morphology of nanoemulsions and is a good way to confirm the results of other analytical methods.

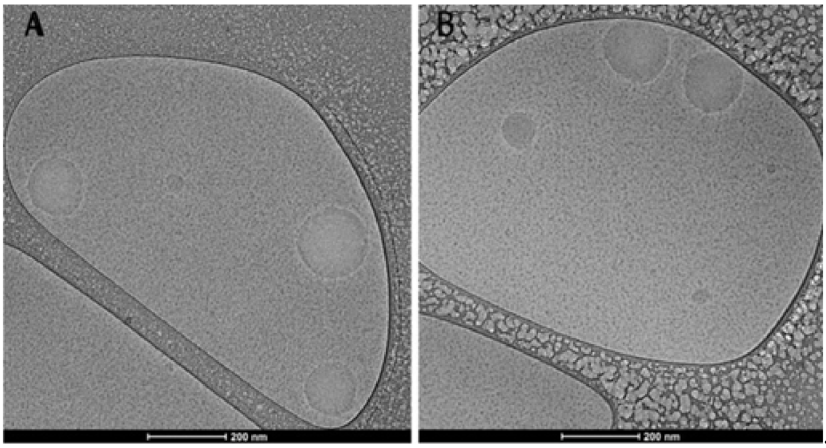

Figure 1: (A) Cym-NE and (B) Myr-NE Cryogenic TEM

In vitro drug release

The ability of nanoemulsions (NEs) to enhance the solubility and bioavailability of pharmaceuticals gives them promise in several domains. The use of NEs has the ability to enhance the solubility of monoterpenes and other less soluble compounds, leading to better drug distribution and efficacy. As shown in Figure 3, nanoemulsions often had a higher cumulative release than free terpenes.

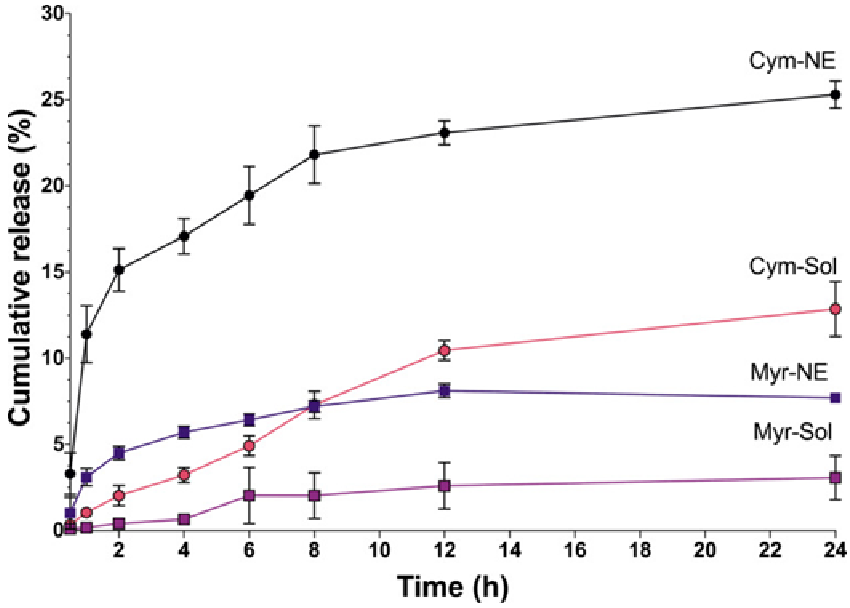

Figure 2: **Free terpenes (Cym-Sol and Myr-Sol) and nanoemulsions (Cym-NE and Myr-NE) for in vitro medication release.**

Properties of NEs that are larvicidal against *Aedes aegypti*

The findings showed that the effectiveness of free myrcene changed when the concentration was increased or decreased. The behaviour and apparent ease of spreading in water of nanoemulsions were superior to those of free myrcene at all concentrations. The mortality rates varied from 10.6% ± 2.3% at 5 mg/L to 100% at 50 mg/L, as shown in Table 5. The effects were more noticeable at lower dosages.

Table 2: **Larvae of *Aedes aegypti* After 24 hours of exposure, the average death rate for free monoterpenes and their nanoemulsion**

		Cym-free	Cym-NE	Myr-free	Myr-NE
Mortality rate after 24 hours on average	**5 mg/L**	20 ± 4	14.6 ± 2.3	13.3 ± 2.3	10.6 ± 2.3
	25 mg/L	83 ± 2.3	78.6 ± 4.6	81.3 ± 4.6	94.6 ± 2.3
	50 mg/L	98.6 ± 2.3	100 ± 0	98.6 ± 2.3	100 ± 0

NEs Cytotoxicity in human keratinocytes

To ensure a drug is compatible with human cells, it must undergo toxicity testing. Table 6 shows the results of the terpene compositions' tests on the HaCAT cell line. The results showed that nanoemulsions exhibited less cytotoxicity than free terpenes, with lower IC50 values.

Table 3: Free terpenes (Cym-free and Myr-free), NEs (Cym-NE and Myr-NE), and surfactants

solution (B-Cym and B-Myr) Inhibitory concentrations 50 (IC_{50}) in human keratinocytes

	Cym-free	Myr-free	B-Cym	B-Myr	Cym-NE	Myr-NE
IC_{50} (mg/mL)	6.43 ± 0.56	1.86 ± 0.15	16.98 ± 0.90	22.37 ± 0.32	14.95 ± 0.64	2.37 ± 0.33

LNCs Acute toxicity using *Galleria mellonella* in vivo model in alternative

Each nanoemulsion (NE) was evaluated for its in vivo toxicity using Galleria mellonella larvae as test animals. No symptoms of acute toxicity were found at dosages ranging from 250 to 1000 mg/kg, as shown in Figure 3, as no deaths were reported. On the other hand, larvae exhibited a fleeting pain response and irregular behaviours such recurrent hopping on the first day after NE injection. Following a five-minute interval, this action stopped.

On the other hand, larvae exposed to free terpenes, especially at greater concentrations, created an oily, sticky web by day's end, which remained until day two. Consistent with other studies, our results show that G. mellonella larvae do not experience acute toxicity when exposed to nanoparticles. In general, it seems that the studied concentrations of NEs are well-tolerated.

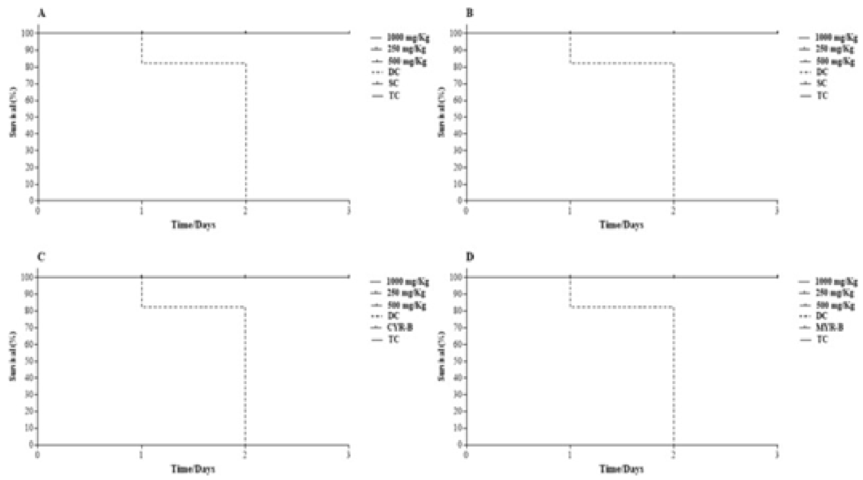

Figure 3: The survival rates of Galleria mellonella larvae subjected to different treatments are shown by the Kaplan-Meier curve. Conditions A, B, C, and D represent Cym-Sol, Myr-Sol, Cym-NE, and Myr-NE, respectively. To guarantee fair comparisons of treatment effects, the research also included control groups: DC (death control, 100% methanol), SC (solvent control, 5% ethanol), and TC (trauma control).

CONCLUSION

Last but not least, bioassays showed that terpene nanoemulsions are just as effective as free terpenes in killing insects, and they could even help terpenes disperse in water. The significant potential for the creation of environmentally benign and effective pest control strategies is presented by the larvicidal activity, safety, and sustained release features of the nanoemulsions. In order to fully use these formulations as an integrated pest management component, more study is needed to optimise them.

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