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ORIGINAL PAPER

Lantana camara L. essential oil mediated nano-emulsion formulation for biocontrol application: anti-mosquitocidal, anti-microbial and antioxidant assay

Vino Udappusamy[1] · Harshavardhan Mohan[2] · Rajan Thinagaran[1]

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Abstract

Mosquitoes play an important role in the spread of vector-borne diseases and their management is highly essential. Plant extracts have been explored for their mosquitocidal activity against different types of vectors. The present work aimed to determine the larvicidal and pupicidal activity of *Lantana camara* L. essential oil-loaded nano-emulsion formulation for the control of pests. The synthesized essential oil-loaded nano-emulsion was subjected to evaluate the antioxidant potential and mosquito larvicidal properties. GC–MS analysis revealed that the essential oil of *Lantana camara* L. leaf contained 12 bioactive components. Caryophyllene oxide (15.81), n-Hexadecanoic acid (4.22), Davanone (6.49) and beta-Sesquiphellandrene (2.32) are the major compounds identified. The nano-emulsion was effective against *A. aegypti* immature stage (larvae and pupae) and adult mosquitoes in laboratory conditions. The LC_{50} was found to be 18.183 ppm (I), 23.337 ppm (II), 29.731 ppm (III), 38.943 ppm (IV) instars and 45.295 ppm (pupae), respectively. The LD_{50} and LD_{90} values for adult mosquitoes were 11.947 mg/cm^2 and 47.716 mg/cm^2, respectively. The antioxidant activity of ascorbic acid (55.9%), glutathione (67.7%) and quercetin (48.6%) was recorded, respectively. The level of acetylcholinesterase (0.06 mM) and alkaline phosphatase (0.05 mM) activity significantly decreased from the control (0.12 mM) which revealed the efficacy of essential oil-loaded nano-emulsion to treat larvae. This study suggested that using an essential oil-loaded nano-emulsion formulation effectively controlled the mosquito vectors. It was also evidenced that the use of nano-emulsion has a great role in near future, especially in vector management.

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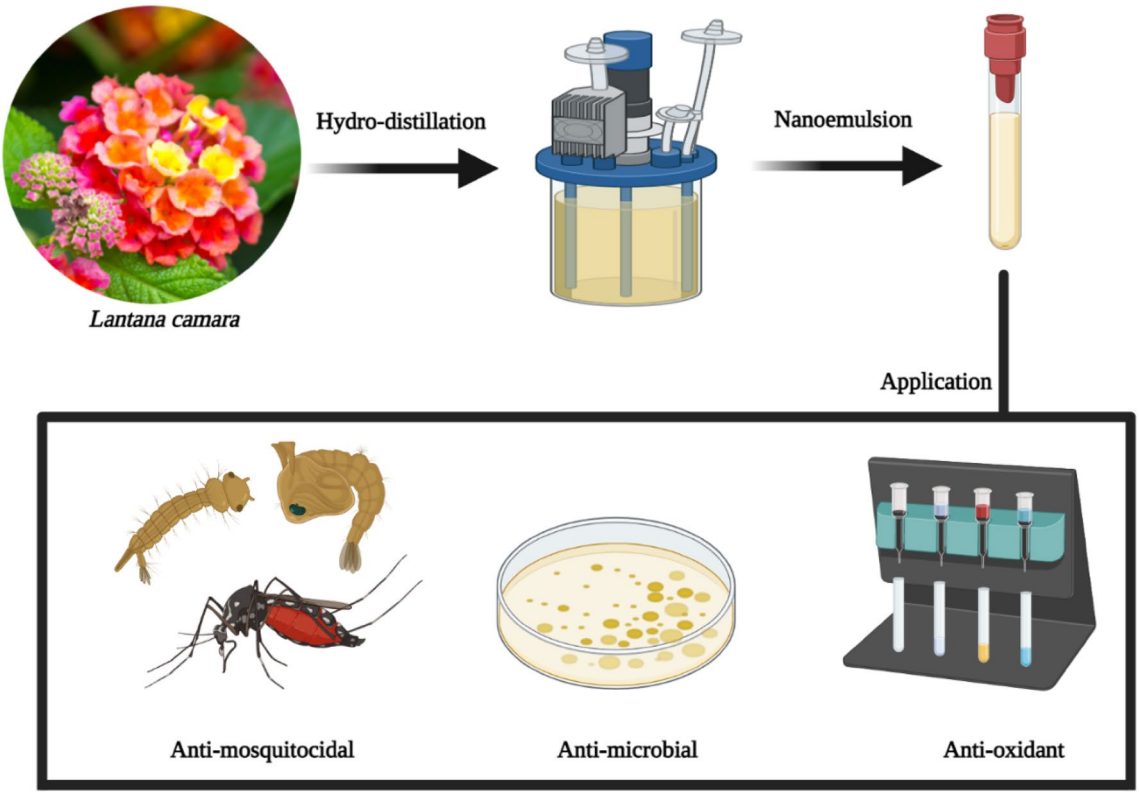
✉ Harshavardhan Mohan
harsha.jia@gmail.com

✉ Rajan Thinagaran
drrajan2012@gmail.com

[1] Department of Biochemistry, PSG College of Arts and Science, Tamil Nadu, Coimbatore 641014, India

[2] Department of Chemistry, Research Institute of Physics and Chemistry, Jeonbuk National University, Jeonju 54896, Republic of Korea

Graphical abstract



Keywords *Lantana camara* L. · Essential oil · Nanoemulsion · Anti-mosquitocidal, antioxidant

Introduction

The *Aedes aegypti* mosquito (Linnaeus 1762) is the primary vector of numerous neglected and developing tropical diseases. It has also become a major public health concern since it transmits many human viral illnesses such as dengue, chikungunya, malaria, flavivirus, Japanese encephalitis and zika virus (Kovendan and Murugan 2011; Fradin and Day 2002). Avoiding mosquito bites and controlling the vectors that spread these diseases has become the utmost concern to control and manage mosquito-borne conditions; and also, insect repellent chemicals are critical in preventing these diseases. Because of relatively extended protection, synthetic compounds such as N, N-diethyl-m toluamide (DEET) have been utilized as a mosquito repellent in most world regions. However, studies have revealed that the majority of synthetic compounds, such as DEET, cause permanent environmental harm and have a high permeability to the skin (Das et al. 2015; Nuchuchua et al. 2009). Furthermore, due to public concern about traditional synthetic pesticides' health and environmental risks, researchers and environmentalists have focused on creating biodegradable Phyto-pesticides to combat dangerous agricultural and public health pests (Maurya et al. 2021).

Plants have potent secondary metabolites including essential oils which are considered to be significant mosquito control agents. When compared to synthetic pesticides, the EEOs are generated by more than 17,500 species of aromatic plants that offered various benefits, including biodegradability, environmental friendly and a low risk for human and animal health (Chermenskaya et al. 2010; Martins et al. 2021). According to's survey on new botanical larvicides, the *Lamiaceae Martinov* family (1820) has EOs and chemical compounds with higher efficacy in larvicide tests (LC50 100 ppm), reaching 19.7 percent when compared to other families with the efficacy of 14.7 per cent (*Cupressaceae*), 12.3 percent (*Rutaceae*), 11.5 percent (*Apiaceae*) and 10.6 per cent (*Myrtaceae*). The primary ingredients of EOs are terpenoids, which are made of low molecular weight monoterpenes and sesquiterpenes, and phenylpropanoids, which are volatile phenolic chemicals (Echeverrí a, J., de Albuquerque. 2019). There are many plant species that are least exploited and one among them is *Lantana camara* L. It belongs to the *Verbenaceae* family and is a notorious

invasive weed reported to be native to tropical, subtropical and temperate regions of more than 60 countries. Extract of *Lantana camara* L. has been used traditionally for centuries to cure various disorders like gastrointestinal, inflammatory, dermatological, rheumatism, fever and headache. Interestingly, different species of *Lantana camara* L. have been reported to contain diverse type and levels of phytochemicals (Mansoori et al. 2020).

Nano-emulsion research has emerged as a possible alternative for intravenous and topical administration. Nano-emulsions are oil-in-water dispersions with a wide range of droplet sizes. (Solans et al. 2003). Nano-emulsions are the method used for controlling mosquitos for a long period (Bouchernal et al. 2004). Owing to this, nanotechnology are considered as a multidisciplinary field used for various applications including insecticidal agent. Nano-emulsions, which are made up of many smaller droplets (20–200 nm) dispersed in the external phase, generally water, and are often stabilised by one or more surfactants, are among the most common nanostructured systems (Benelli et al. 2018). Nano-emulsions are notable for their substantial qualities, which include an increase in physical–chemical properties, improved stability and a rise in biological activities due to an increase in the area of certain surfaces. Furthermore, in vector control methods, aqueous nano-emulsions containing bioactive vegetable oils are a potentially affordable alternative to conventional pesticides. As *Lantana camara* L. was known for their therapeutic properties and least explored, the essential oil was prepared using its leaves and is nano-formulated to increase its efficacy as a bio-pesticidal agent. The main objective of this study was to develop an ideal nano-formulation from the essential oil obtained from the leaves of *Lantana camara* L. and their mosquitocidal activity was evaluated against *Aedes aegypti*.

Materials and methods

Extraction of essential oil from leaves of Lantana camara L.

The essential oil extraction from leaves of *Lantana camara* L. was performed by hydro-distillation using a Clevenger apparatus according to the procedure of Fernandes et al. (2013). A clean, sterile, dark glass bottle was used to store the extracted essential oil and preserved at 4 °C for further use.

Preparation of nano-emulsion formulation

The nano-emulsion formulation was screened based on the protocol of Ostertag et al. (2012). Briefly, the nano-emulsion was prepared with basic oil composition and Tween-20 and then it was stirred at 750 rpm using a magnetic stirrer for 15 min. Followed by stirring, water was added drop by drop at a flow rate of 5 mL/minute. This mixture was stirred at 1200 rpm for 30 min.

Nano-emulsion physicochemical characterization

The physicochemical evaluation was also carried out to elucidate the optimal parameters for the essential oil-loaded nano-capsules (Oliveira et al. 2017).

Particle size distribution of nano-emulsion

Particle size analysis (PSA) is an essential technique for evaluating the size and homogeneity of the nano-emulsion dispersed droplets. The average PSA and polydispersity index (PDI) of *Lantana camara* L. nano-emulsion oil were studied using a Photon Correlation Spectroscopy (Malvern Ze-easier 3000 HS) (Harleen Kaur et al. 2020).

UV–Visible spectra and fourier transform infrared spectroscopy analysis

The synthesized nano-emulsions were subjected to UV–Vis spectral analysis with a wavelength range of 300 to 450 nm to validate the synthesized nano-emulsion. The presence of functional groups in synthesized nano-emulsion was detected using Fourier transform infrared (FT-IR) spectra (Perkin Elmer Spectrum RXI FT-IR spectrophotometer) with scan range of 4000–400 cm^{-1} (Natrajan et al. 2015).

Scanning electron microscopy

The nano-emulsion formulation was characterized for its shape and surface morphology using a scanning electron microscope (JSM-6360, JEOL, Japan) at 15 kV. Briefly, about 5 mL of sample was freeze-dried and examined (Songkro et al. 2012, Sundararajan et al. 2018).

Gas chromatography–mass spectrometry (GC–MS) analysis

The GC–MS analysis for prepared nano-emulsion was performed through electron impact mode with an ionization voltage of 70 eV, an injector temperature of 230 °C, and a detector temperature of 280 °C. The carrier gas used was helium (99.9% purity) at a 1 mL/minute flow rate, and approximately 1 mL of sample was injected. The oven temperature was set to 80 °C (isothermal for 5 min, then to 200 °C at 5 °C/minutes, and finally to 280 °C at 5 °C/minutes (isothermal for 16 min). The identification of compounds from spectral data was compared with the standards

available in mass spectral records (NIST and WILEY libraries).

Mosquito culture

The *Aedes aegypti* were cultured under laboratory conditions (26 ± 2 °C, 70–85% RRH 14:10 (L:D) photoperiod) and placed in 22 × 10 × 8 cm plastic containers containing 1000 mL of tap water for larval hatching. The larvae were reared in plastic containers and were fed dog biscuits and yeast in a 3:1 ratio (w/w). The breeding container was checked, and the water was changed periodically. The containers were kept closed with a cloth to prevent the entry of adult mosquito. Both larvae and pupae were collected based on the standard protocol described by Sundararajan et al. 2018 and Ramar et al. (2013).

Larvicidal and pupicidal activity of nano-emulsion

The prepared nano-emulsion formulations were tested with five different concentrations including 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm. The 25 numbers of I to IV instar of *A.aegypti* larvae and pupae were introduced into a 500 mL glass beaker containing 249 mL of dechlorinated water and 1 mL of desired nano-emulsion concentration according to the standard procedure of nano-emulsion bio-pesticide. The larva was exposed to de-chlorinated water without bio-pesticide served as control. The Tempos of 1 ppm and concentration were used as a reference standard for comparative analysis. The mortality rate was calculated using Abbott's formula (Abbott's 1925).

$$Mortality(\%) = \left[\frac{Number\ of\ larve/pupae\ dead}{Number\ of\ larve/pupae\ released}\right] \times 100 \quad (1)$$

$$Corrected\ moratality(\%) = \left[\frac{Mortality\ in\ test - mortality\ in\ control}{100 - Mortality\ in\ control}\right] \times 100 \quad (2)$$

Adulticidal activity

An adulticidal activity was carried out as per WHO standard protocol. Briefly, the nano-emulsion essential oil of *Lantana camara* L. impregnated filter paper was prepared by adding 2 mL of the nano-emulsion formulation. The different doses viz., 10, 20, 30, 40 and 50 mg/cm^2 were studied. The fixed doses of 2 mL of formulation were applied on Whatman no. 1 filter papers (size 12 × 15 cm^2), including a control set. Two to 5 day-old sugar-fed mosquitoes ($N = 20$) were exposed to pre-treated Whatman no. 1 filter paper for 60 min, and mosquito rate was recorded every 5 min. After 1 hour, knocked down mosquitoes were transferred into recovery test tubes for mortality observation at 24th hour. A comparative study was done using 0.05% deltamethrin insecticide. The lethal dose (LD_{50} and LD_{90}) was calculated by probit analysis using SPSS software.

Antioxidant activity

The ABTS + stock solution was prepared by mixing 5 mL of 7 mM (2, 2'-and-bis- (3-ethylbenzothiazoline—6- sulfonic acid) of ABTS radical scavenging activity of the extract was measured using the method described by Rice Evans et al. (1997). The standard stock solution, i.e., ascorbic acid, glutathione and quercetin was prepared in acetone. The total antioxidant activity was calculated using the following formula:

$$Total\ antioxidant\ activity(\%) = \left[\frac{O.D\ ABTS + O.D\ Sample}{O\ DABTS}\right] \times 100 \quad (3)$$

Acetylcholinesterase activity

The acetylcholinesterase activity in the larvae was measured according to the protocol described earlier by Ikezawa and Taguchi (1981). Briefly, 100 µl of homogenate mixture was mixed uniformly with 500 µl of PBS (100 mM, pH 7.4), 100 µl of 15 mM 5–5-dithiobis 2-nitro benzoic acid***** and 100 µl of 15 mM acetylcholine iodide whereby it acts as the substrate. The mixture was incubated at room temperature for 3 min, and the optical density was measured at 600 nm.

Alkaline phosphatase activity

The alkaline phosphatase activity of the homogenized larvae was measured by the method of Asakura, (1978). Briefly, 100 µl of homogenized larvae was used with 500 µl of 15 mM Tris–HCl buffer (pH 8), and 500 µl of 15 mM p-nitrophenyl phosphate was added. The homogenized mixture was incubated for 3 min at 35 °C in water bath. Then this homogenized mixture was treated with 100 µl of NaOH to stop the enzymatic reaction. Finally, the mixtures were centrifuged for 5 min and the optical density of the supernatant was measured.

Antimicrobial properties

Three gram-positive bacteria, *Staphylococcus aureus* (ATCC- 12,493), *Streptococcus pneumonia* (ATCC-49,619), *Staphylococcus epidermis* (ATCC- 12,228)

and two gram-negative bacteria, *Klebsiella pneumonia* (ATCC- 33,495) and *Salmonella typhi* (ATCC- 14,028), were selected for the study. The disc diffusion method was performed for *Lantana camara* L. nano-emulsion oil at different concentration ranges, 100, 200, 300, 400 µg/mL and the antibiotic, erythromycin (10 µg/mL) was used as a positive control, while *Lantana camara* L. nano-emulsion oil (100 µg/mL) served as a negative control. Then, the plates were incubated at 37ºC for 24 h. The sizes of the inhibitory zones were measured in millimetres (mm).

Statistical analysis

All the data were subjected to Analysis of Variance (ANOVA). The $LC_{50,}$ $LC_{90,}$ $LD_{50,}$ LD_{90} values and their 95% confidence limits were subjected to Probit analysis. The Chi-Square test was used to determine the model's goodness of fit. An AP value of less than 0.05 was regarded as a substantial divergence from the data model. In the event of a large deviation, the 90 per cent confidence limit for LC50 and LC90 was calculated using a heterogeneity factor. SPSS Software version 16 was used for all of the analysis. For the importance of discrepancies between values, a probability value of $P < 0.05$ was utilized.

Results

Preparation and physicochemical characterization of nano-emulsion

Nano-emulsion was prepared using 75% (w/w) of water, 15% (w/w) of essential oil and 10% (w/w) of Tween 20 (Fig. 1).

Particle size distribution of nano-emulsion

It was confirmed that the average droplet size of nano-emulsion was 418.4 ± 17 nm (Fig. 1a). The current results showed a significant decrease in the dimensions of the nano-emulsion formulation. The mean droplet size of 18.4 ± 0.004), which was in concurrence with the formation of nano-emulsions.

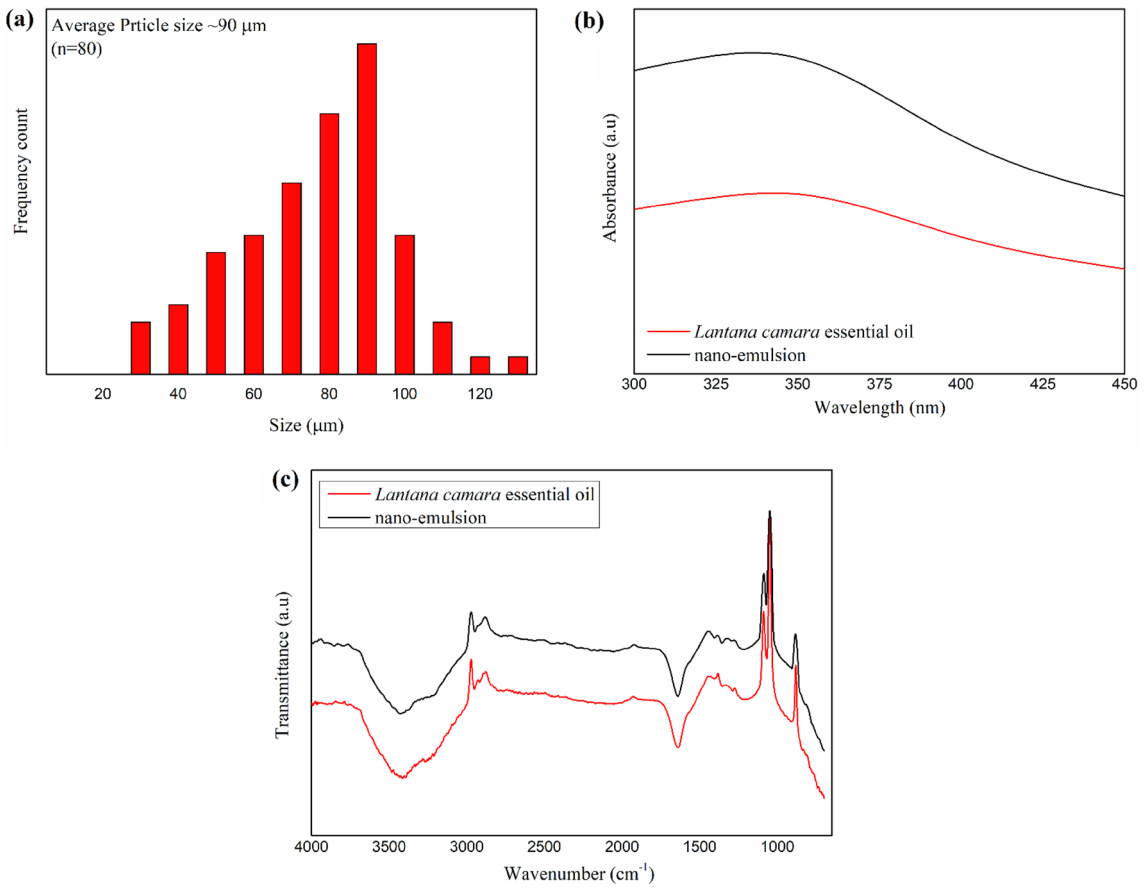


Fig. 1 **a** Particle size distribution of a nanoemulsion, **b** UV–vis spectra of essential oil and loaded nano-emulsion and **c** FTIR spectra of essential oil and loaded nano-emulsion

UV analysis and fourier transformed infrared (FTIR) spectroscopy analysis

Figure 1b shows the UV–Vis spectra of the synthesized nano-emulsion oil. The absorption maxima of *Lantana camara* L. essential oil and nano-emulsion oil were observed at range of 325 and 375 nm, respectively. The interface of major functional groups involved in the essential oil and nano-emulsion formulation was analysed through FTIR spectroscopy (Fig. 1c). Both essential oil and nano-emulsified essential oil have similar functional groups which confer the presence of essential oil and nano-emulsified essential oil. The broad absorption band was noticed at 3415 cm^{-1} and 3427 cm^{-1} depicting the presence of O–H hydroxyl group, the C = C group was noted at 1641 cm^{-1}, 1224 cm^{-1} and 1217 cm^{-1} conferred C–O–C ether group, 2810 cm^{-1} denoted aliphatic alkane group with C-H stretching and 973 cm^{-1} has C = C bending with alkene as a major constituent.

Scanning electron microscopy (SEM)

The morphology of the essential oil and essential oil-loaded nano-emulsion of *Lantana camara* L. was studied by using SEM and it was inferred that essential oil was found to have crystalline morphology, whereas nano-emulsion had rosette-like morphology (Fig. 2). The difference exhibited here is due to the difference in the preparation of sample.

Gas chromatography–mass spectrometry analysis of Lantana camara L. essential oil

In addition, we used GC–MS to identify the bioactive compounds found in the nano-emulsion essential oil of *Lantana camara* L. (Table 1). It was revealed that caryophyllene oxide, a major phyto-constituent present in the nano-emulsion essential oil, was the reason for the larvicidal activity.

Larvicidal and pupicidal activity

Larval and pupal mortality of formulated nano-emulsion essential oil of *Lantana camara* L. was observed. Table 2 provides the results of larval and pupal mortality for first to fourth-instar larvae at different concentrations (10, 20, 30, 40 and 50 ppm). About 38.30% mortality was noted at first instar larvae with 10 ppm concentration, whereas it has been increased to 94.60% at 50 ppm. The mortality rate for second to fourth and pupa was found to be 31.20%, 26.80%, 19.50%, 12.20% for 10 ppm concentration, respectively, and at 50 ppm concentration the mortality rate for fourth and pupa was observed as 85.72%, 72.40%, 61.60%, 53.60%, respectively. The LC50 and LC90 values

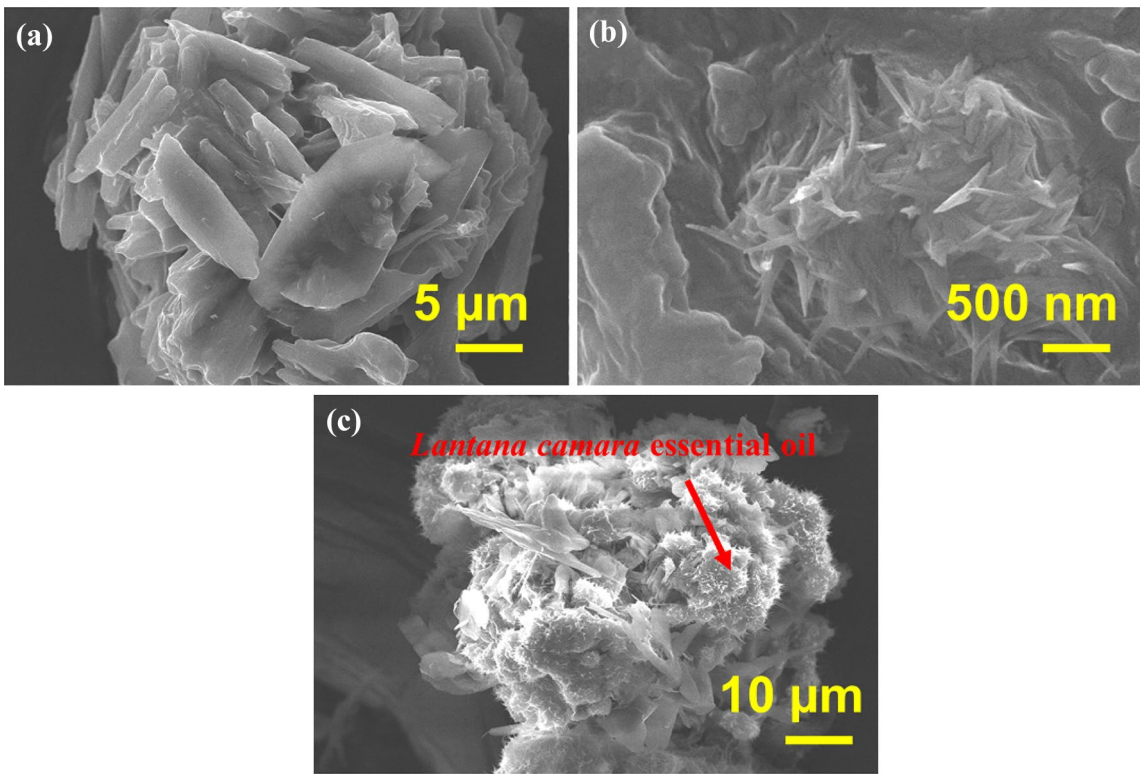


Fig. 2 SEM of **a** essential oil and (**b**, **c**) loaded nano-emulsion

Table 1 Compounds identified in the Plant sample *Lantana camara*. *L* Essential oil

No	RT (min)	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	9.061	Humulene	$C_{15}H_{24}$	204.35 g/mol	2.91
2	9.876	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42 g/mol	4.22
3	11.035	alpha -Caryophyllene	$C_{15}H_{24}$	204.35 g/mol	6.14
4	11.45	Eucalyptol	$C_{10}H_{18}O$	154.25 g/mol	0.37
5	14.366	α-Pinene	$C_{10}H_{16}$	136.23 g/mol	0.51
6	15.098	Isoborneol	$C_{10}H_{18}O$	154.25 g/mol	2.20
7	16.760	Phytol	$C_{20}H_{40}O$	296.5 g/mol	0.37
8	17.032	Caryophyllene oxide	$C_{15}H_{24}O$	220.35 g/mol	15.81
9	19.11	Butyl Hexyl Phthalate	$C_{18}H_{26}O_4$	306.4 g/mol	0.24
10	20.34	beta-Sesquiphellandrene	$C_{15}H_{24}$	204.35	2.32
11	20.77	Bicyclogermacrene	$C_{15}H_{24}$	204.35	1.09
12	21.11	Davanone	$C_{15}H_{24}O_2$	236.35	6.49

were represented as follows; LC50 value of first instar was 18.183 ppm, second instar was 23.337 ppm, the third instar was 29.731 ppm, fourth instar was 38.943 ppm, and pupa was 45.295 ppm, respectively. The LC90 value of first instar was 46.959 ppm, second instar was 56.482 ppm, third instar was 71.676 ppm, fourth instar was 82.017 ppm and of pupae was 85.991 ppm, respectively.

Adulticidal activity

The adulticidal activity was conducted to test the efficacy of nano-emulsion on adult mosquitoes of *A. aegypti*. The percentage of mortality was calculated for the adult dengue vector, *A. aegypti* for different doses of emulsion that include 10, 20, 30, 40 and 50 mg/cm^2. The mortality was increased with the increased dosage concentration. The adult mortality was 48.2% at 10 mg/cm^2, whereas it was increased to 92.5% at 50 mg/cm^{2}dose, respectively (Table 3). The LD_{50} and LD_{90} values (Table 2) of the nano-emulsion treated paper were found to be effective against *A. aegypti* (LD_{50} = 11.947 mg/cm^2 and LD_{90} =47.716 mg/cm^2), respectively. Significant mortality was observed after the treatment of emulsion formulation against the vector mosquito of *A. aegypti*. There was no mortality recorded in the control. X^2 value was significant at the $p \leq 0.05$ level.

Antioxidant activity

The antioxidant activity by absorbance spectrum of ABTS + at dose-dependent method results was present (Fig. 3). The activity for standards of ascorbic acid (55.9%), glutathione (67.7%) and quercetin (48.6%) showed inhibition at 50 mM concentration, respectively.

Biochemical activity (acetylcholinesterase and alkaline phosphatase)

The nano-emulsion treated acetylcholinesterase and alkaline phosphatase assays were studied in the IV instar larvae of *A.aegypti* (Fig. 4). In the control larvae, acetylcholinesterase activity was 0.3241 M, and acetylcholinesterase released minutes/mg of protein. The exposed larvae to the nano-emulsion formulation at 50 ppm eventually lowered to 0.06 M acetylcholinesterase released minutes/mg of protein in the treated larvae.

Antimicrobial activity

The antimicrobial efficacy of *Lantana camara* L. nano-emulsion oil revealed that they are highly effective against *Staphylococcus aureus* and *Staphylococcus epidermis*. It was also found that gram-positive bacteria are more susceptible than that of gram-negative bacteria (Table 4 and Fig. 5).

Discussion

Tropical diseases are transmitted through vectors considered a serious public health problem in developing countries. *Aedes aegypti* is a major vector responsible for transmitting arboviruses and causes dengue, chikungunya, zika, etc., which have high mortality rates. Synthetic chemicals have been a prominent tool for combating the vectors that cause serious illness. Yet these synthetic chemicals are hazardous to the environment. It was known that plants exhibited various pharmacological properties and are traditionally used as insecticidal agents for controlling the mosquito. Among natural products, essential oils are a significant pharmacological agent that is least toxic to the environment and has high lipophilicity, making them an excellent alternative

Table 2 The percentage larval mortality (mean ± SE) and lethal concentrations (LC_{50}, LC_{90})of *Aedes aegypti* in different exposure periods with Nano-emulsion oil formulation

Instars	Larval and pupal mortality (%) (Mean ± S.D)					LC_{50} (LC_{90})	95% Confidence limit		Regression equation	Chi square value (χ^2)
	Concentration (ppm)									
	10	20	30	40	50		LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)		
I	38.3 ± 1.98	52.4 ± 1.85	67.2 ± 1.72	81.8 ± 2.03	94.6 ± 3.13	18.183 (46.959)	14.406–21.169	42.633–53.154	$X = 0.045\ Y = -0.810$	1.705*
II	31.2 ± 1.16	44.5 ± 1.73	59.1 ± 3.13	73.6 ± 2.05	85.72 ± 2.07	23.337 (56.482)	19.718–26.402	50.689–65.209	$X = 0.039\ Y = -0.902$	0.157*
III	26.8 ± 1.16	39.89 ± 2.25	47.95 ± 1.48	64.48 ± 1.00	72.4 ± 2.15	29.731 (71.676)	25.851–33.573	62.227–87.648	$X = 0.031\ Y = -0.908$	0.579
IV	19.5 ± 2.52	28.3 ± 2.71	38.8 ± 0.74	53.6 ± 1.62	61.6 ± 3.77	38.943 (82.017)	34.943–44.267	70.387–102.269	$X = 0.030\ Y = -1.159$	0.319*
Pupa	12.2 ± 1.72	21.7 ± 1.98	32.2 ± 1.60	45.8 ± 2.13	53.6 ± 1.85	45.295 (85.991)	40.85–51.886	73.915–106.877	$X = 0.031\ Y = -1.426$	0.592*

Mortality rates are means ± SD of five replicates. No mortality was observed in the control. Within each column means followed by the same letter(s) are not significantly different ($P < 0.05$). *LC50* lethal concentration that kills 50% of the exposed organisms, *LC90* lethal concentration that kills 90% of the exposed organisms, *LCL* lower confidence limit, *UCL* upper confidence limit, $\chi 2$ Chi- square test, *NS* not significant

* Indicates significance

Table 3 The percentage of adult mortality (mean ± SE) and lethal doses (LD_{50}, LD_{90}) of *Aedes aegypti* after 24 h with nano-emulsion formulation

Mosquito species	Concentration (mg/cm^2)	Mortality (%) (mean ± SD)	LD_{50} ppm (*LFL-UFL*)	LD_{90} ppm (*LFL-UFL*)	χ^2
A.aegypyti	10	48.2 ± 1.21[a]	11.947 (5.524–16.293)	47.716 (42.433–55.949)	0.419*
	20	61.6 ± 1.72[b]			
	30	72.2 ± 2.28[c]			
	40	83.7 ± 1.04[d]			
	50	92.5 ± 3.32[e]			

Mortality rates are means ± SD of five replicates. No mortality was observed in the control. Within each column means followed by the same letter(s) are not significantly different ($P < 0.05$). *LC_{50}* lethal concentration that kills 50% of the exposed organisms, *LC_{90}* lethal concentration that kills 90% of the exposed organisms, *LCL* lower confidence limit, *UCL* upper confidence limit. Chi-square value followed by an * is significant (heterogeneity factor used in calculation of confidence limits) ($P < 0.05$)

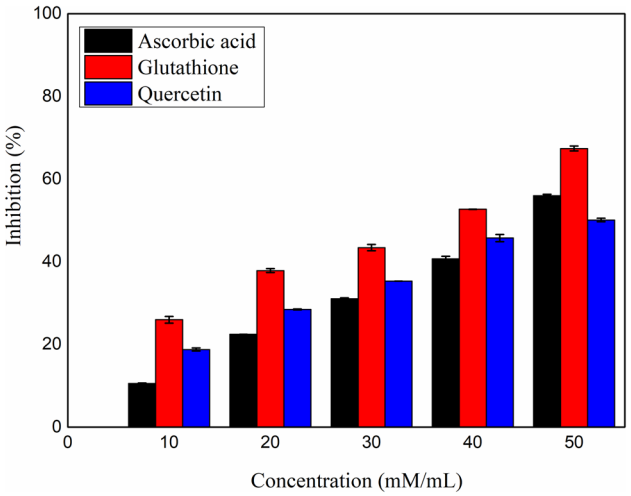


Fig. 3 Percentage inhibition ABTS + with proposition of dose defendant concentration of standard antioxidants

to synthetic pesticides. Various studies have demonstrated that essential oils possess prominent larvicidal and repellent effects on different species of insects (Faustino et al. 2020).

Lantana camara L. is an aromatic shrub and widely distributed species among the *Lantana* genus. They are native to tropical and sub-tropical regions of America and are used as ornamental plants in different parts of the world. It is used to treat various diseases and disorders. In India, essential oil from the leaves of *Lantana camara* L. is extracted and used as an antiseptic and antifungal agent (Dougnon and Ito 2020). Despite their significant pharmacological properties, the mosquitocidal properties and antioxidant activity of essential oil from the leaves of *Lantana camara* L. are least explored. Hence an attempt was made in this study to

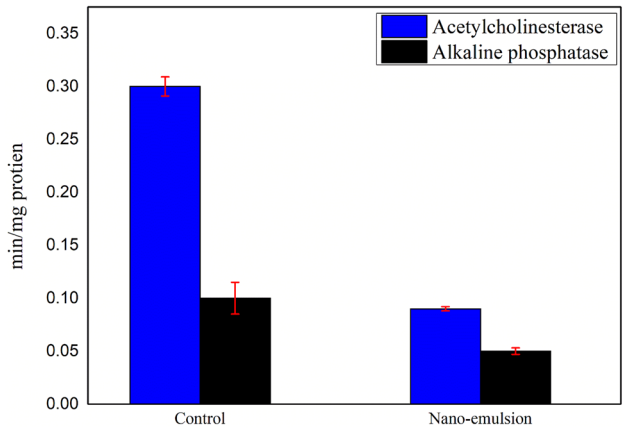


Fig. 4 Biochemical assays of treated and control IV instar larval

Table 4 Antimicrobial activity of *Lantana camara* nano-emulsion oil (100 µg /ml) by agar well- diffusion assay

Human pathogenic bacteria	Zone of inhibition in (mm)				Erythromycin 10 µg/ml
	100	200	300	400	
Staphylococcus aureus	14	18	21	23	13
Streptococcus pneumonia	11	14	17	18	18
Staphylococcus epidermis	18	20	23	25	13
Klebsiella pneumonia	8	10	11	22	9
Salmonella typhi	12	15	17	21	15

determine the efficacy of essential oil in controlling mosquito vectors. When using the essential oil as a pesticide, the efficacy will not be long lasting. In order to overcome the hurdles, a nano-emulsion formulation of essential oil was made whereby it bypasses the technological limitations like hydrophobicity, reactivity and volatility of biological compounds (Sugumar et al. 2014). Therefore, the present study aimed to evaluate the mosquitocidal and antioxidant activity of the formulated nano-emulsified essential oil of *Lantana camara* L.

Nano-emulsion was prepared using 75% (w/w) of water, 15% (w/w) of essential oil and 10% (w/w) of Tween 20. It was noted that the average droplet size of nano-emulsion was found to be 418.4 ± 17 nm. The absorption maxima of *Lantana camara* L. essential oil and nano-emulsion oil was observed at a range of 325 and 375 nm, respectively. Both essential oil and nano-emulsified essential oil have similar functional groups like hydroxyl, ether, alkane, alkene groups which confers the major functional constituents of essential oil and nano-emulsified essential oil. The morphology of the essential oil and essential oil-loaded nano-emulsion of *Lantana camara* L. was studied by using SEM and it was inferred that essential oil was found to have crystalline morphology whereas nano-emulsion had rosette like morphology. The major bioactive compound found in the nano-emulsion essential oil of *Lantana camara* L. was caryophyllene oxide which was the reason for larvicidal activity. Larval, pupal, adulticidal and antioxidant activity of formulated nano-emulsion essential oil of *Lantana camara* L. was observed and found to have significant mosquitocidal activity. The antimicrobial efficacy of *Lantana camara* L. nano-emulsion oil revealed that they are highly effective against *Staphylococcus aureus* and *Staphylococcus epidermis*.

All these results are well correlated with the research findings of Fernandes et al. (2013) and Conti et al. (2010) who reported that *R. officinalis* essential oil employed in the study is known to exhibit larvicidal activity. The larvicidal activity of the eucalyptus oil nano-emulsion was compared

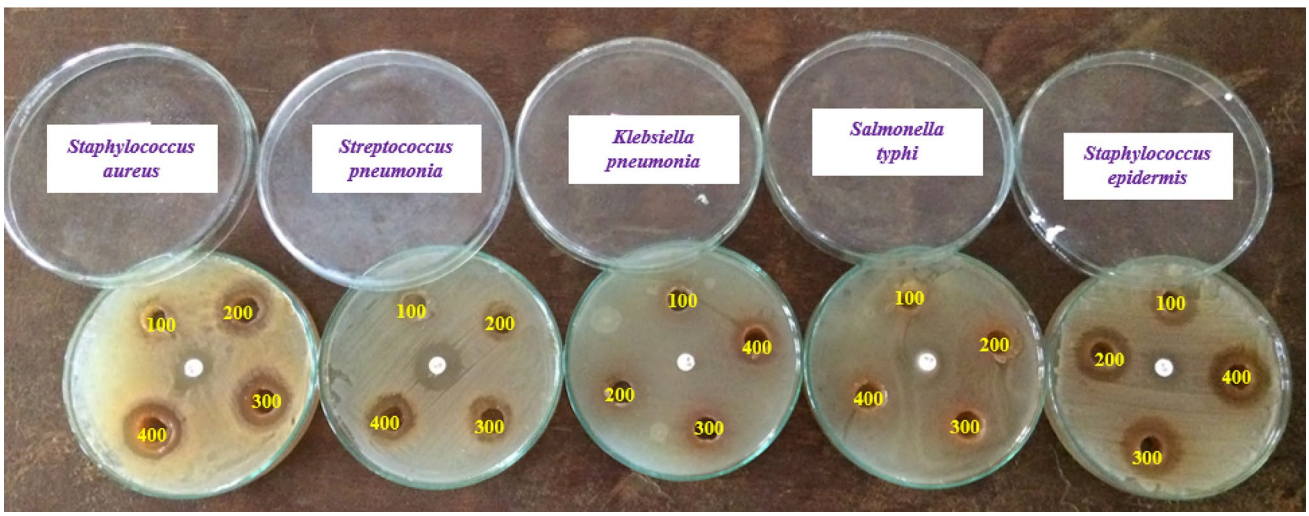


Fig. 5 Antimicrobial activity of various bacterial strains used to treat nano-emulsion oil

with that of the bulk emulsion. The eucalyptus oil nano-emulsion was more effective than the bulk version. The reported histological effects of the eucalyptus oil nano-emulsion on *Cx. quinquefasciatus* were consistent with Suresh Kumar et al. (2013), who found that the midgut content of *Aedes aegypti* treated with nano-permethrin was damaged. The consistency and stability of the nano-emulsion can be determined. The nano-emulsion is inversely proportional to the uniformity and stability of the nano-emulsion (Shinoda and Saito 1969). Aromatic plants and their derivatives are steam distilled to produce essential oils high in bioactive chemicals that degrade to non-toxic molecules. They are utilized as insect repellents since they have no negative health effects and are a safe and environmentally friendly alternative to synthetic pesticides (Isman 2000).

After 2 months, the variation of insecticidal activity of the nano-emulsion of eucalyptus oil with and without the aqueous filtrate against *T. castaneum* was observed. The toxicity of the nano-emulsion prepared with this filtrate as a continuous phase stays unchanged, whereas the insecticidal activity of the nano-emulsion formulations prepared with distilled water decreases significantly. An earlier study reported that LC_{50} values of the formulations, i.e., F1, F2, F3 and F4, indicated that F4 was more toxic than F2 and F3, but F1 was not as harmful as F4, F3 and F2 because of the absence of aqueous filtrate as a continuous phase in the formulation (Pant et al. 2014). It was observed that the nano-emulsion containing essential oil of *R. officinalis* caused $80 \pm 10\%$ of mortality after 24 h and $90 \pm 10\%$ of mortality after 48 h. The particle size of droplets remained in the nano-metric range, so penetration and potential larvicidal activity may not be affected. Further investigations would be necessary to confirm these findings.

After 1 day of preparation, it was observed that some particles were around 10 nm, which was responsible for the modal profile. Further characterization revealed that narrow distribution was achieved, probably due to disintegration and regeneration of micelles. Penetration through the cuticle is crucial for insecticidal activity and is recognized as one of the possible mechanisms of insecticides (Kasai et al. 2014). These aqueous filtrate solid cakes possessing insecticidal properties were used in distilled water to enhance the shelf life of eucalyptus oil for a longer period. Within 24 h, a eucalyptus oil nano-emulsion containing *Karanja* and *jatropha* aqueous filtrate at concentrations of 300 and 1500 ppm eliminated all *T. castaneum* adults. For its broad effectiveness against *T. castaneum*, the nano-emulsion formulation with an average particle size of 77 nm was chosen (Pant et al. 2014). With this background, our results suggest that the nano-emulsion containing 20% (w/w) of *Lantana camara L.* and *Eucalyptus globules* essential oil, 20% (w/w) of emulsifier (w/w) and 45% (w/w) of water can be considered a potential larvicidal and pupicidal agent.

Conclusions

In this present investigation, a prominent result of nano-emulsion using an essential oil-loaded formulation was noticed. There was a considerable decrease in the droplet size distribution of nano-emulsion after formulation preparation. The property of the nano-emulsion was related to the essential oil and ingredient added in the nano-emulsion formulations. The different activities such as larvicidal, pupicidal, adulticidal, antioxidant and anti-enzyme activity of nano-emulsions were evaluated by selected nano-emulsion formulation. The nano-emulsion based essential oil mediated formulation enhanced larvicidal and pupicidal, and an adulticidal action against *A. aegypti* was observed. The antimicrobial activity of *Lantana camara L.* nano-emulsion oil at various concentrations ensures its use in treating infectious illnesses. The current results support the possibility of nano-emulsion in developing essential oil-based novel Nano-pesticide for dengue vector mosquito control. This is an important report assessing the mosquitocidal, antioxidant and anti-enzyme activity for oil loaded nano-emulsion. Further, research is needed to use nano-formulation on non-target organisms, and a toxicity study will be evaluated. It could be explored as a promising nano-pesticide that can be used to control mosquito.

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Declarations

Conflict of interest The author(s) declare(s) that there is no conflict of interest.

References

Bouchemal K, Briançon S, Perrier E, Fessi H (2004) Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimization. Int J Pharm 280(1–2):241–251. https://doi.org/10.1016/j.ijpharm.2004.05.016

Benelli G, Pavela R, Petrelli R, Cappellacci L, Canale A, Senthil-Nathan S, Nicoletti M, Maggic F (2018) Not just popular spices! Essential oils from *Cuminum cyminum* and *Pimpinella anisum* are toxic to insect pests and vectors without affecting non-target invertebrates. Ind Crops Prod 124:236–243. https://doi.org/10.1016/j.indcrop.2018.07.048

Conti B, Canale A, Bertoli A, Gozzini F, Pistelli L (2010) Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus (Diptera: Culicidae)*. Parasitol Res 107(6):1455–1461. https://doi.org/10.1007/s00436-010-2018-4

Chermenskaya TD, Stepanycheva EA, Shchenikova AV, Chakaeva AS (2010) Insectoacaricidal and deterrent activities of extracts of *Kyrgyzstan* plants against three agricultural pests. Ind Crops Prod 32(2):157–163. https://doi.org/10.1016/j.indcrop.2010.04.009

Das NG, Dhiman S, Talukdar PK, Rabha B, Goswami D, Veer V (2015) Synergistic mosquito-repellent activity of *Curcuma longa, Pogostemon heyneanus* and *Zanthoxylum limonella* essential oils. J Infect Public Health 8(4):323–328. https://doi.org/10.1016/j.jiph.2015.02.005

Dougnon G, Ito M (2020) Sedative effects of the essential oil from the leaves of *Lantana camara* occurring in the Republic of Benin via inhalation in mice. J Nat Med 74(1):159–169. https://doi.org/10.1007/s11418-019-01358-9

Echeverría J, de Albuquerque RDDG (2019) Nanoemulsions of essential oils: new tool for control of vector-borne diseases and in vitro effects on some parasitic agents. Medicines. 6:1–11. https://doi.org/10.3390/medicines6020042

Fernandes CP, Mascarenhas MP, Zibetti FM, Lima BG, Oliveira RP, Rocha L, Falcão DQ (2013) HLB value, an important parameter for the development of essential oil phytopharmaceuticals. Rev Bras Farmacogn 23(1):108–114. https://doi.org/10.1590/S0102-695X2012005000127

Faustino CG, de Medeiros FA, Ribeiro Galardo AK, Lobato Rodrigues AB, Lopes Martins R, de MedeirosSouzaLima Y, Fechine Tavares J, de AlvesMedeiros MA, Dos SantosCruz J, Almeida S (2020) Larvicide activity on *Aedes aegypti* of essential oil nanoemulsion from the *Protium heptaphyllum* Resin. Molecules (Basel, Switzerland) 25(22):5333. https://doi.org/10.3390/molecules25225333

Fradin MS, Day JF (2002) Comparative efficacy of insect repellents against mosquito bites. NEJM 347(1):13–18. https://doi.org/10.1056/nejmoa011699

Isman MB (2000) Plant essential oils for pest and disease management. Crop Prot 19(8–10):603–608. https://doi.org/10.1016/S0261-2194(00)00079-X

Kaur H, Pancham P, Kaur R, Agarwal S, Singh M (2020) Synthesis and characterization of *Citrus limonum* essential oil based nanoemulsion and its enhanced antioxidant activity with stability for transdermal application. J Biomater Nanobiotechnol 11(4):215–236. https://doi.org/10.4236/jbnb.2020.114014

Kasai S, Komagata O, Itokawa K, Shono T, Ng LC, Kobayashi M, Tomita T (2014) Mechanisms of pyrethroid resistance in the dengue mosquito vector, *Aedesaegypti*: target site insensitivity, penetration, and metabolism. PLOS Neglect Trop Dis 8:1–23. https://doi.org/10.1371/journal.pntd.0002948

Kumar RS, Shiny PJ, Anjali CH, Jerobin J, Goshen KM, Magdassi S, Chandrasekaran N (2013) Distinctive effects of nano-sized permethrin in the environment. Environ Sci Pollut Rec 20(4):2593–2602. https://doi.org/10.1007/s11356-012-1161-0

Kovendan K, Murugan K (2011) Effect of medicinal plants on the mosquito vectors from the different agro-climatic regions of Tamil Nadu India. Adv Environ Biol 5(2):335–344 (**ISSN 1995-0756**)

Mansoori A, Singh N, Dubey SK, Thakur TK, Alkan N, Das SN, Kumar A (2020) Phytochemical characterization and assessment of crude extracts from Lantana camara L. for antioxidant and antimicrobial activity. Front Agron. https://doi.org/10.3389/fagro.2020.582268

Martins RL, Rodrigues ABL, de Menezes RÉ, Santos LL, Brandão LB, Faustino CG, Galardo AKR (2021) Development of larvicide nanoemulsion from the essential oil of Aeollanthus suaveolens Mart. ex Spreng against Aedes aegypti, and its toxicity in non-target organism. Arab J Chem 14(6):103148. https://doi.org/10.1016/j.arabjc.2021.103148

Natrajan D, Srinivasan S, Sundar K, Ravindran A (2015) Formulation of essential oil-loaded chitosan–alginate nanocapsules. J Food Drug Anal 23(3):560–568. https://doi.org/10.1016/j.jfda.2015.01.001

Nuchuchua O, Sakulku U, Uawongyart N, Puttipipatkhachorn S, Soottitantawat A, Ruktanonchai U (2009) *In vitro* characterization and mosquito (*Aedes aegypti*) repellent activity of essential-oils-loaded nanoemulsions. AAPS PharmSciTech 10(4):1234–1242. https://doi.org/10.1208/s12249-009-9323-1

Ostertag F, Weiss J, McClements DJ (2012) Low-energy formation of edible nanoemulsions: factors influencing droplet size produced by emulsion phase inversion. J Colloid Interface Sci 388(1):95–102. https://doi.org/10.1016/j.jcis.2012.07.089

Oliveira AE, Bezerra DC, Duarte JL, Cruz RA, Souto RN, Ferreira RM, Fernandes CP (2017) Essential oil from *Pterodon emarginatus* as a promising natural raw material for larvicidal nanoemulsions against a tropical disease vector. Sustain Chem Pharm 6:1–9. https://doi.org/10.1016/j.scp.2017.06.001

Pant M, Dubey S, Patanjali PK, Naik SN, Sharma S (2014) Insecticidal activity of *eucalyptus* oil nanoemulsion with *karanja* and *jatropha* aqueous filtrates. Int Biodeterior Biodegradation 91:119–127. https://doi.org/10.1016/j.ibiod.2013.11.019

Shinoda K, Saito H (1969) The stability of O/W type emulsions as functions of temperature and the HLB of emulsifiers: the emulsification by PIT-method. J Colloid Interface Sci 30(2):258–263. https://doi.org/10.1016/S0021-9797(69)80012-3

Solans S, Esquena J, Forigianini A (2003) Absorption and aggregation of surfactants in solution. Nanoemulsion: formation properties and applications. Marcel Dekker, New York, pp 525–554

Songkro S, Hayook N, Jaisawang J, Maneenuan D, Chuchome T, Kaewnopparat N (2012) Investigation of inclusion complexes of *citronella oil, citronellal and citronellol* with *β-cyclodextrin* for mosquito repellent. Journal of J Incl Phenom Macrocycl Chem 72(3):339–355. https://doi.org/10.1007/s10847-011-9985-7

Sugumar S, Clarke SK, Nirmala MJ, Tyagi BK, Mukherjee A, Chandrasekaran N (2014) Nanoemulsion of *eucalyptus* oil and its larvicidal activity against *Culex quinquefasciatus*. Bull Entomol Res 104(3):393–402. https://doi.org/10.1017/S0007485313000710

Sundararajan B, Moola AK, Vivek K, Kumari BR (2018) Formulation of nanoemulsion from leaves essential oil of *Ocimum basilicum L.* and its antibacterial, antioxidant and larvicidal activities (*Culex quinquefasciatus*). Microb Pathog 125:475–485. https://doi.org/10.1016/j.micpath.2018.10.017