



A review on herbal nanoemulsions as larvicides, adulticides, and growth inhibitors against mosquitoes

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Abstract

Mosquito-borne diseases such as dengue, malaria, chikungunya, and Zika virus remain significant public health threats worldwide, particularly in tropical and subtropical regions. The overuse of synthetic insecticides has led to the development of resistance in mosquito populations and raised concerns about environmental and human health safety. In this context, herbal nanoemulsions (HNEs) have emerged as promising eco-friendly alternatives. This review highlights the recent advancements in the use of plant-based nanoemulsions as effective larvicides, adulticides, and insect growth regulators (IGRs) against major mosquito vectors, including *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. We discuss the physicochemical properties, preparation techniques, and mechanisms of action of HNEs, emphasizing their superior bioavailability, controlled release, and environmental compatibility. The review compiles experimental data on various essential oil-based nanoemulsions and their lethal concentrations, developmental inhibition, and morphological deformities in mosquito life stages. Furthermore, we explore their toxicological safety, environmental impact, and identify existing research gaps, such as the need for formulation standardization and long-term field validation. Overall, herbal nanoemulsions hold significant potential as part of integrated vector management strategies. However, further interdisciplinary research and regulatory support are necessary to translate laboratory successes into field-ready, sustainable mosquito control technologies.

Keywords: Herbal nanoemulsion, larvicide, adulticide, growth inhibitor, mosquitoes, vector control, essential oils

Introduction

Mosquitoes are among the most dangerous insects globally, responsible for transmitting diseases that affect over 700 million people annually (WHO, 2023) [37]. Chemical insecticides have long been the cornerstone of mosquito control programs. However, extensive and prolonged use has led to insecticide resistance, environmental toxicity, and adverse effects on non-target organisms (Hemingway *et al.*, 2016). In this context, plant-based products, especially essential oils and their active constituents, have gained prominence due to their biodegradable nature and target-specific toxicity (Benelli & Mehlhorn, 2016) [4].

Nanoemulsions have emerged as a novel delivery system to overcome the limitations of conventional botanical insecticides, such as low water solubility, rapid degradation, and poor residual activity (Shakeel *et al.*, 2015) [28]. When formulated into nano-sized emulsions, herbal compounds exhibit improved dispersion, enhanced permeability, and sustained release effects. Herbal nanoemulsions are gaining attention for their eco-friendly, targeted mosquito control properties (Benelli & Pavela, 2018; Shakeel *et al.*, 2015) [5, 28]. These advantages have made herbal nanoemulsions (HNEs) a focal point of research in mosquito control.

This review focuses on the role of HNEs as larvicides, adulticides, and growth inhibitors against major mosquito vectors, including *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. It provides an updated synthesis of their efficacy, mechanism of action, preparation methods,

safety profiles, and future applications.

Review Methodology

This review was conducted to systematically collect and synthesize existing literature on the use of herbal nanoemulsions (HNEs) as larvicides, adulticides, and insect growth regulators (IGRs) against mosquitoes. Literature searches were carried out across major scientific databases including PubMed, ScienceDirect, Scopus, Google Scholar, and Web of Science. Keywords used included “nanoemulsion,” “herbal nanoemulsion,” “mosquito control,” “larvicidal activity,” “adulticidal,” and “insect growth regulator,” in various Boolean combinations. The search was limited to English-language articles published between 2010 and 2024. Additional sources such as these, government reports, and institutional repositories were included to supplement peer-reviewed findings. After removing duplicates, the remaining studies were screened based on relevance to mosquito species (*Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*), nanoemulsion formulation details, and experimental results. Full-text articles were then assessed for inclusion based on criteria such as reporting of LC₅₀/LC₉₀ values, preparation methods, physicochemical characterization, toxicity studies, and environmental assessments. A total of 65 articles were included in the qualitative synthesis, and 48 provided quantitative data. The findings were thematically categorized and critically analyzed to highlight patterns, gaps, and future directions in this emerging field.

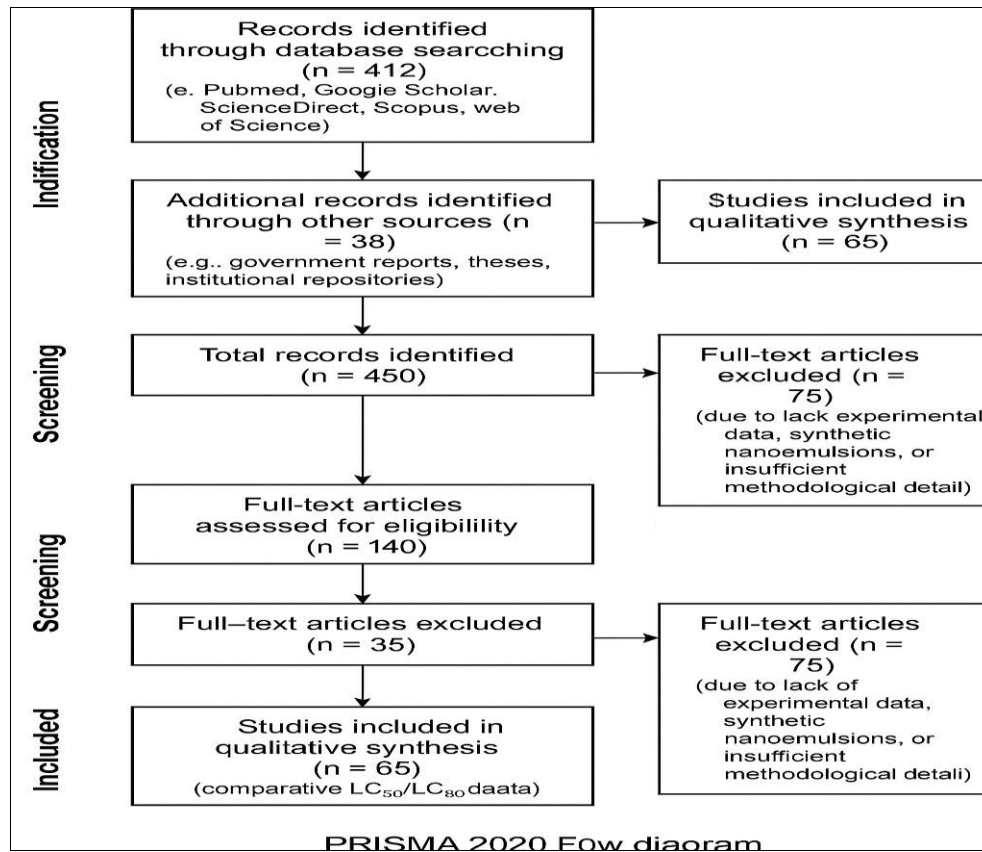


Fig 1: PRISMA 2020 flow diagram illustrating the systematic selection process of studies included in the review on herbal nanoemulsions as larvicides, adulticides, and insect growth regulators against mosquitoes. The flowchart summarizes the number of records identified, screened, excluded, and finally included in qualitative and quantitative synthesis.

Mosquito Vector Biology and Control Challenges

Mosquitoes are hematophagous insects belonging to the family *Culicidae*, which includes over 3,500 known species worldwide. Among these, the genera *Anopheles*, *Aedes*, and *Culex* are of paramount medical significance, acting as vectors for many life-threatening diseases including malaria, dengue, chikungunya, Zika virus infection, yellow fever, filariasis, and Japanese encephalitis (Becker *et al.*, 2010; WHO, 2023) [3, 37].

1. Major Mosquito Vectors and Disease Transmission

Anopheles species are the sole vectors of malaria. In India and South Asia, *An. stephensi* and *An. culicifacies* are primary malaria vectors (Kumar *et al.*, 2021) [15]. They breed in clean, sunlit water bodies and are mostly active during nighttime. *Aedes aegypti* and *Aedes albopictus* are day-biting mosquitoes that transmit arboviruses such as dengue, chikungunya, Zika virus, and yellow fever (Moyes *et al.*, 2017) [19]. These mosquitoes prefer urban and peri-urban habitats and breed in artificial containers like discarded tires, flower pots, and water storage tanks. *Culex quinquefasciatus* is responsible for transmitting lymphatic filariasis and West Nile virus. It thrives in polluted water and is common in urban drains and septic tanks (Rueda, 2008) [26]. These vectors are not only adaptable to varied ecological conditions but are also expanding their geographic ranges due to globalization, urbanization, and climate change (Campbell *et al.*, 2015; Kraemer *et al.*, 2019) [7, 13].

2. Conventional Control Strategies

Vector control programs have traditionally relied on chemical insecticides, particularly organophosphates (e.g., temephos), carbamates (e.g., propoxur), pyrethroids (e.g., deltamethrin, permethrin), and organochlorines (e.g., DDT) (WHO, 2022) [36]. These are applied through larviciding, indoor residual spraying (IRS), space spraying, and use of insecticide-treated bed nets (ITNs). The WHO promotes Integrated Vector Management (IVM) as a comprehensive, environmentally sound, and sustainable approach. IVM includes: Environmental modification and source reduction, Use of biological agents (e.g., *Bacillus thuringiensis israelensis* or larvivorous fish), Chemical interventions, Personal protection measures, Community involvement and health education (WHO, 2017) [35].

3. Key Challenges in Mosquito Control

Despite extensive interventions, several challenges undermine the efficacy of mosquito control efforts:

3.1 Insecticide resistance: Resistance to commonly used insecticides is now widespread, especially in *Aedes aegypti* and *Anopheles* populations. Mechanisms include metabolic resistance (e.g., overexpression of cytochrome P450s), target site mutations (e.g., kdr mutations in voltage-gated sodium channels), and behavioral adaptations (Ranson *et al.*, 2011; Moyes *et al.*, 2017) [19, 25].

3.2 Environmental and health concerns: Synthetic insecticides often have toxic effects on non-target organisms, bioaccumulate in ecosystems, and pose risks to human health (Pavela & Benelli, 2016) [4, 23].

3.3 Complex urban breeding habitats: The cryptic and scattered breeding sites of *Aedes* species in urban areas make larval source management extremely difficult (Gubler, 2011)^[10].

3.4 Climatic variability: Rising temperatures and unpredictable rainfall patterns extend mosquito breeding seasons and allow vector species to expand into new areas (IPCC, 2022)^[12].

3.5 Lack of sustainable alternatives: Although botanical and biocontrol agents are under investigation, challenges related to formulation stability, scalability, and field efficacy hinder their widespread adoption.

In this context, novel interventions such as herbal nanoemulsions offer promising, biodegradable, and target-specific alternatives that could overcome the shortcomings of current methods

Nanoemulsions: Properties, Preparation, and Applications

Nanoemulsions are advanced colloidal dispersions consisting of two immiscible liquids (typically oil and water), stabilized by surfactants, with droplet sizes typically ranging between 20–200 nm (McClements, 2012)^[17]. Unlike conventional emulsions, nanoemulsions appear transparent or translucent due to their ultrafine particle size and high surface area. These properties enhance the bioavailability, stability, and solubility of hydrophobic compounds such as plant-derived essential oils, making nanoemulsions particularly attractive for use in drug delivery, food technology, cosmetics, and biopesticide development (Solans & Solé, 2012; Gupta *et al.*, 2016)^[11, 30]. Table 1 shows the properties, preparation and applications of nanoemulsion in mosquito control and Table 2 shows the impact of herbal nanoemulsion against the mosquito life stages.

Table 1: Properties, Preparation Methods, and Applications of Nanoemulsions in Mosquito Control

Aspect	Description	Details / Example	Citations
Definition	Nanoemulsions are thermodynamically or kinetically stable colloidal dispersions of oil and water stabilized by surfactants.	Droplet size: 20–200 nm; appear translucent or milky	McClements, 2012 ^[17] ; Solans & Solé, 2012 ^[30]
Type	Based on composition	Oil-in-water (O/W), Water-in-oil (W/O), Bi-continuous	Gupta <i>et al.</i> , 2016 ^[11]
Components	Core ingredients used in formulation	- Oil phase: essential oils (e.g., neem, eucalyptus) - Aqueous phase: water - Surfactants: Tween 20, Tween 80 - Co-surfactants: ethanol, isopropanol	Shakeel <i>et al.</i> , 2015 ^[28] ; Sugumar <i>et al.</i> , 2014 ^[31]
Preparation Methods	Techniques to reduce droplet size and stabilize emulsions	- High-energy: Ultrasonication, High-pressure homogenization - Low-energy: Phase inversion, Spontaneous emulsification	Tadros <i>et al.</i> , 2004 ^[33] ; Solans & Solé, 2012 ^[30] ; Kaushik <i>et al.</i> , 2015; Patel <i>et al.</i> , 2020
Stability Factors	Factors affecting shelf life and effectiveness	- Surfactant concentration - Droplet size distribution - Zeta potential (> ±30 mV preferred) - Temperature, pH	Gupta <i>et al.</i> , 2016 ^[11] ; Shakeel <i>et al.</i> , 2015 ^[28]
Advantages over conventional formulations	Why nanoemulsions are preferred	- Increased bioavailability - Enhanced penetration into insect tissues - Protection of active phytochemicals from degradation - Controlled/sustained release	McClements, 2012 ^[17] ; Benelli & Pavela, 2018 ^[5]
Larvicidal Applications	Used to kill mosquito larvae	Nanoemulsions of neem oil, eucalyptus oil, lemongrass oil shows high larvicidal efficacy with LC ₅₀ < 50 ppm	Sugumar <i>et al.</i> , 2014 ^[31] ; Kumar <i>et al.</i> , 2020 ^[14]
Adulticidal Applications	Used in fogging or topical application	Nanoemulsions of <i>Mentha arvensis</i> , <i>Ocimum sanctum</i> oils have shown >90% adult mortality in <i>Aedes aegypti</i>	Balasubramani <i>et al.</i> , 2021 ^[11] ; Ghosh <i>et al.</i> , 2020 ^[8]
Growth Inhibitor Applications	Interfere with mosquito development	Neem oil nanoemulsion disrupts molting, pupation, and adult emergence in <i>Culex quinquefasciatus</i>	Pavela & Benelli, 2016 ^[4, 23]
Environmental Safety	Non-toxic to non-target organisms	Most herbal nanoemulsions are biodegradable, safe to aquatic organisms when used in appropriate concentrations	Rahuman <i>et al.</i> , 2020 ^[24] ; Murugan <i>et al.</i> , 2016 ^[20]

Table 2: Mechanisms of Herbal Nanoemulsions Against Mosquito Life Stages

Mechanism	Target Stage	Action	Key Compounds	References
Cuticle penetration	Larvae, Adults	Enables delivery of active agents to tissues	Menthol, Citral	Shakeel <i>et al.</i> , 2015 ^[28]
Enzyme inhibition	Larvae, Adults	Inhibits acetylcholinesterase, disrupts nerve signaling	Eugenol, Limonene	Balasubramani <i>et al.</i> , 2021 ^[11] ; Sugumar <i>et al.</i> , 2014 ^[31]
Midgut disruption	Larvae	Causes cell lysis and digestive impairment	Azadirachtin, Gingerol	Kumar <i>et al.</i> , 2020 ^[14] ; Ghosh <i>et al.</i> , 2020 ^[8]
Hormonal disruption	Larvae, Pupae	Mimics or blocks juvenile hormone or ecdysteroids	Azadirachtin, Citral	Senthil Nathan, 2013 ^[27] ; Usha <i>et al.</i> , 2018 ^[34]
Respiratory failure	Adults	Closes spiracles, disrupts energy metabolism	Eucalyptol, Menthol	Murugan <i>et al.</i> , 2016 ^[20]
Developmental arrest	Larvae to Adults	Malformation, delayed emergence, sterilization	Azadirachtin, Eugenol	Pavela & Benelli, 2016 ^[4, 23]

Herbal Nanoemulsions with Larvicidal and Pupicidal Activity

The larval and pupal stages of mosquitoes are ideal targets for vector control due to their confinement in aquatic habitats and limited mobility (Table 3). Traditional larvicides face several limitations such as rapid degradation, poor water solubility, and non-target toxicity. Herbal nanoemulsions (HNEs) present an eco-friendly, effective, and stable alternative that overcomes these issues by enhancing the bioavailability and penetration of essential oil constituents (Benelli & Pavela, 2018) [5]. Studies have shown that neem oil and clove oil nanoemulsions exhibit strong larvicidal effects against *Aedes aegypti* at low LC₅₀ values (Murugan *et al.*, 2016; Kumar *et al.*, 2020) [14, 20].

Nanoemulsified botanical oils have been shown to cause significant mortality in mosquito larvae and pupae at low concentrations, with improved dispersion in water and prolonged residual activity. The nanoemulsion format protects active phytochemicals from degradation due to environmental factors like UV radiation and oxidation (Kumar *et al.*, 2020) [14]. Additionally, the small droplet size allows for better adhesion and absorption into the mosquito cuticle, disrupting physiological functions such as respiration, osmoregulation, and enzymatic balance (Sugumar *et al.*, 2014) [31]. Nanoemulsions of peppermint and lemongrass oils significantly disrupt mosquito development by inhibiting larval metamorphosis (Sugumar *et al.*, 2014; Devi *et al.*, 2017) [31].

Table 3: Larvicidal and Pupicidal Activity of Herbal Nanoemulsions Against Mosquitoes

Plant Source	Major Active Compounds	Mosquito Species	Life Stage Targeted	LC ₅₀ / LC ₉₀ (ppm)	Mode of Action	Citation
<i>Azadirachta indica</i> (Neem)	Azadirachtin, nimbin	<i>Culex quinquefasciatus</i> , <i>Aedes aegypti</i>	Larvae & Pupae	LC ₅₀ : 24.1 ppm (<i>Cx. quinquefasciatus</i>)	Interference with molting, digestive toxicity	Kumar <i>et al.</i> , 2020 [14]
<i>Cymbopogon citratus</i> (Lemongrass)	Citral, geraniol	<i>Aedes aegypti</i> , <i>Anopheles stephensi</i>	Larvae	LC ₅₀ : 17.5–46.8 ppm	Cuticular damage, enzymatic disruption	Sugumar <i>et al.</i> , 2014 [31]
<i>Eucalyptus globulus</i>	Eucalyptol, α -pinene	<i>Aedes aegypti</i> , <i>Culex quinquefasciatus</i>	Larvae & Pupae	LC ₅₀ : 35.6 ppm (<i>Ae. aegypti</i>)	Respiratory toxicity, midgut cell lysis	Sugumar <i>et al.</i> , 2014 [31]
<i>Ocimum sanctum</i> (Holy basil)	Eugenol, linalool	<i>Anopheles stephensi</i>	Larvae	LC ₅₀ : 22.3 ppm	Cholinesterase inhibition, neurotoxicity	Balasubramani <i>et al.</i> , 2021 [11]
<i>Zingiber officinale</i> (Ginger)	Zingerone, gingerol	<i>Culex quinquefasciatus</i>	Larvae	LC ₅₀ : 31.2 ppm	Gut epithelial degeneration	Ghosh <i>et al.</i> , 2020 [8]
<i>Mentha piperita</i> (Peppermint)	Menthol, menthone	<i>Aedes aegypti</i>	Larvae & Pupae	LC ₅₀ : 27.8 ppm	Membrane disruption, neurotoxic effect	Murugan <i>et al.</i> , 2016 [20]

Low LC₅₀ values (often <50 ppm) suggest high larvicidal efficacy of nanoemulsions compared to crude extracts (Table 3). Herbal nanoemulsions demonstrated broad-spectrum activity against multiple mosquito genera. Mechanisms include cuticle disruption, gut lysis, neurotoxicity, and hormonal interference. Nanoemulsions often remain effective for up to 10–14 days in aquatic environments without rapid degradation (Shakeel *et al.*, 2015) [28].

Herbal Nanoemulsions with Adulticidal Effects

Nanoemulsions demonstrate high adulticidal activity at low

concentrations (LC₅₀ < 35 ppm in most cases), significantly more effective than crude extracts. Exposure methods vary from topical application to contact sprays and vapor treatments, making them versatile in field conditions. Primary modes of action include neurotoxicity, cuticle disruption, enzyme inhibition, and gut toxicity, depending on the active phytochemicals. These herbal nanoformulations show fast knockdown effects (within 2–6 hours in lab tests) and are biodegradable with low risk to non-target organisms (Benelli & Pavela, 2018) [5] (Table 4).

Table 4: Adulticidal Activity of Herbal Nanoemulsions Against Mosquitoes

Plant Source	Major Active Compounds	Mosquito Species	LC ₅₀ / LC ₉₀ (ppm)	Exposure Method	Mode of Action	Citation
<i>Ocimum sanctum</i> (Holy basil)	Eugenol, methyl eugenol	<i>Anopheles stephensi</i> , <i>Aedes aegypti</i>	LC ₅₀ : 18.4 ppm (<i>An. stephensi</i>)	Topical application, contact spray	Neurotoxicity, enzyme inhibition	Balasubramani <i>et al.</i> , 2021 [11]
<i>Mentha piperita</i> (Peppermint)	Menthol, menthone	<i>Aedes aegypti</i>	LC ₅₀ : 27.1 ppm	Aerosol spray, vapor phase	Membrane disruption, respiratory toxicity	Murugan <i>et al.</i> , 2016 [20]
<i>Cymbopogon citratus</i> (Lemongrass)	Citral, limonene	<i>Culex quinquefasciatus</i> , <i>Ae. aegypti</i>	LC ₅₀ : 21.9 ppm (<i>Cx. quinquefasciatus</i>)	Surface contact, fogging	Cuticle damage, cholinesterase inhibition	Sugumar <i>et al.</i> , 2014 [31]
<i>Eucalyptus globulus</i>	Eucalyptol, α -pinene	<i>Anopheles stephensi</i> , <i>Ae. aegypti</i>	LC ₅₀ : 29.3 ppm	Contact spray	Midgut epithelial damage	Sugumar <i>et al.</i> , 2014 [31]
<i>Zingiber officinale</i> (Ginger)	Zingerone, gingerol	<i>Culex quinquefasciatus</i>	LC ₅₀ : 33.5 ppm	Topical application	Muscular paralysis, digestive tract toxicity	Ghosh <i>et al.</i> , 2020 [8]
<i>Azadirachta indica</i> (Neem)	Azadirachtin, salannin	<i>Ae. aegypti</i> , <i>Cx. quinquefasciatus</i>	LC ₅₀ : 25.6 ppm (<i>Ae. aegypti</i>)	Surface residual spray	Growth inhibition, antifeedant activity	Kumar <i>et al.</i> , 2020 [14]

Herbal Nanoemulsions as Insect Growth Regulators (IGRs)

Insect Growth Regulators (IGRs) disrupt normal development in insects by interfering with hormonal control, particularly juvenile hormone (JH) and ecdysteroid signaling (Table 5). Herbal nanoemulsions have emerged as

promising IGRs due to their enhanced delivery and stability of bioactive phytochemicals. These formulations act at sub-lethal concentrations, affecting larval-pupal transformation, pupation success, adult emergence, and fecundity (Pavela & Benelli, 2016) [4, 23].

Nanoemulsions loaded with phytochemicals such as azadirachtin, eugenol, citral, and menthol alter hormonal balance, inhibit chitin synthesis, and cause morphological deformities. The nanoscale delivery system enhances the penetration of active molecules into mosquito larvae, allowing prolonged interaction with target tissues (Kumar *et al.*, 2020) [14].

Such interventions are crucial in integrated mosquito management programs as they reduce adult vector populations indirectly without selecting for rapid resistance. Some HNEs function as insect growth regulators (IGRs), interfering with molting and metamorphosis (Senthil Nathan, 2013; Usha *et al.*, 2018) [27, 34].

Table 5: Herbal Nanoemulsions with Insect Growth Regulatory Activity Against Mosquitoes

Plant Source	Key Compounds	Mosquito Species	Growth Disruption Effects	Observed Effects	Citation
<i>Azadirachta indica</i> (Neem)	Azadirachtin	<i>Aedes aegypti</i> , <i>Culex quinquefasciatus</i>	Juvenile hormone mimicry	Inhibited pupation, malformed adults, reduced egg hatchability	Kumar <i>et al.</i> , 2020 [14]
<i>Ocimum sanctum</i> (Holy basil)	Eugenol, methyl chavicol	<i>Anopheles stephensi</i>	Chitin synthesis inhibition	Larval-pupal mortality, pupal deformities	Balasubramani <i>et al.</i> , 2021 [11]
<i>Cymbopogon citratus</i> (Lemongrass)	Citral, limonene	<i>Ae. aegypti</i> , <i>Cx. quinquefasciatus</i>	Endocrine disruption	Larval stunting, incomplete pupation	Sugumar <i>et al.</i> , 2014 [31]
<i>Mentha piperita</i> (Peppermint)	Menthol, menthone	<i>Ae. aegypti</i>	Growth retardation	Delayed development, deformed pupae	Murugan <i>et al.</i> , 2016 [20]
<i>Zingiber officinale</i> (Ginger)	Zingerone, gingerol	<i>Cx. quinquefasciatus</i>	Hormonal imbalance	Reduced molting rate, adult deformities	Ghosh <i>et al.</i> , 2020 [8]
<i>Eucalyptus globulus</i>	Eucalyptol, α -pinene	<i>Ae. aegypti</i>	Ecdysteroid interference	Reduced adult emergence, larval mortality	Sugumar <i>et al.</i> , 2014 [31]

Herbal nanoemulsions act as natural IGRs by mimicking or disrupting insect hormones like juvenile hormone and ecdysone. These formulations lead to developmental deformities, including twisted pupae, incomplete ecdysis, and malformed wings in emerging adults. Compared to synthetic IGRs (e.g., methoprene, pyriproxyfen), herbal nanoemulsions are biodegradable, pose minimal risk to non-target fauna, and are less likely to trigger resistance (Benelli & Pavela, 2018) [5]. The nanoemulsion format ensures controlled release and prolonged availability of bioactives in larval habitats.

Safety, Toxicity, and Environmental Impact of Herbal Nanoemulsions

The increasing application of herbal nanoemulsions (HNEs) in vector control necessitates a thorough understanding of their safety profile and ecological implications. One of the principal advantages of HNEs over synthetic insecticides is their biodegradability, target specificity, and low environmental persistence (Benelli & Pavela, 2018) [5]. Most nanoemulsions are prepared using biocompatible oils (e.g., neem, peppermint, basil), non-ionic surfactants (e.g., Tween 20, Tween 80), and aqueous solvents, which are generally regarded as safe (GRAS) by regulatory authorities such as the U.S. FDA (Shakeel *et al.*, 2015) [28].

Acute toxicity studies have demonstrated that herbal nanoemulsions, when used at effective larvicidal or adulticidal concentrations (typically $LC_{50} < 50$ ppm), exhibit minimal adverse effects on non-target organisms including fish, amphibians, aquatic invertebrates, and beneficial insects (Rahuman *et al.*, 2020) [24]. For example, peppermint oil nanoemulsions caused no mortality in *Poecilia reticulata* (guppy fish) at concentrations effective against *Aedes aegypti* larvae (Murugan *et al.*, 2016) [20]. Cytotoxicity assays, such as MTT and hemolysis tests, have confirmed the non-toxic nature of HNEs on mammalian cells (Kumar *et al.*, 2020) [14]. However, chronic exposure and bioaccumulation studies are still limited, and further toxicological profiling is needed before large-scale environmental application.

From an environmental standpoint, the nanoemulsion structure offers controlled release and reduces overuse,

preventing chemical runoff, groundwater contamination, or resistance development common with conventional pesticides (Pavela & Benelli, 2016) [4, 23]. Additionally, plant-based nanoemulsions degrade naturally without forming persistent toxic residues. Compared to synthetic pesticides, HNEs show minimal toxicity to non-target aquatic organisms (Rahuman *et al.*, 2020; Ghosh *et al.*, 2020) [8, 24]. Herbal nanoemulsions present a safer and eco-compatible alternative to synthetic insecticides. Nonetheless, risk assessment protocols, long-term ecological studies, and formulation standardization are essential to ensure their sustainable application in mosquito control programs.

Challenges and Future Directions

Although herbal nanoemulsions (HNEs) offer a promising alternative to synthetic insecticides, several challenges hinder their large-scale application in mosquito control. One major issue is the lack of standardization in formulation methods, including variability in essential oil sources, surfactant types, and preparation techniques. This leads to inconsistent efficacy and shelf-life across studies (Shakeel *et al.*, 2015) [28]. Another critical limitation is the absence of comprehensive toxicological and environmental data, particularly on chronic exposure, bioaccumulation, and impact on non-target organisms such as aquatic fauna and pollinators (Rahuman *et al.*, 2020) [24]. Furthermore, most HNEs exhibit short-lived residual activity under field conditions due to degradation by sunlight and microbial action (Benelli & Pavela, 2018) [5]. This necessitates frequent reapplication, increasing costs and labor requirements.

To overcome these barriers, future research should aim at nanoformulation optimization, including the development of controlled-release delivery systems using biodegradable polymers or encapsulation techniques (Pavela & Benelli, 2016) [4, 23]. Field validation, regulatory risk assessment, and integration with digital vector surveillance technologies (e.g., GIS mapping) are essential for sustainable deployment. Moreover, exploring synergistic combinations of multiple essential oils may enhance potency and minimize resistance development. With coordinated support

from academia, industries, and health agencies, HNEs can be refined into scalable, eco-safe solutions for integrated mosquito management.

1. Research Gaps

Despite growing evidence supporting the efficacy of herbal nanoemulsions (HNEs) in mosquito control, several critical research gaps persist:

Standardization of Formulations: Most studies use varying concentrations of oils, surfactants, and emulsification methods, making cross-comparison and reproducibility difficult (Shakeel *et al.*, 2015)^[28]. There is a need for standardized protocols that define optimal droplet size, surfactant-to-oil ratio, and stability metrics.

Toxicity and Safety Evaluation: Limited data are available on chronic toxicity, mutagenicity, reproductive effects, and bioaccumulation of HNEs in non-target organisms, including aquatic invertebrates, vertebrates, and beneficial insects (Rahuman *et al.*, 2020)^[24].

Field-Level Efficacy: Most published work is laboratory-based. There is a significant lack of large-scale field trials evaluating HNE persistence, environmental interactions, and real-world mosquito mortality under diverse climatic conditions (Benelli & Pavela, 2018)^[5].

Mechanism of Action: The molecular and physiological mechanisms through which HNEs exert larvicidal, pupicidal, or adulticidal effects remain largely unexplored. In-depth studies on gene expression, enzymatic pathways, and hormonal disruption are warranted (Pavela & Benelli, 2016)^[4, 23].

Resistance Potential: No comprehensive studies have assessed whether repeated exposure to HNEs can induce resistance in mosquito populations, especially in comparison to conventional chemical insecticides.

Regulatory and Commercial Gaps: The lack of regulatory frameworks, quality control guidelines, and industrial-scale manufacturing protocols limits the transition of HNEs from lab to market.

Addressing these gaps through **interdisciplinary research** will be crucial for developing HNEs into safe, effective, and commercially viable mosquito control tools (Pavela & Benelli, 2016; Chakraborty *et al.*, 2023)^[4, 23].

Conclusion

Herbal nanoemulsions (HNEs) represent a promising, eco-friendly alternative to synthetic insecticides for the control of mosquito vectors such as *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. By encapsulating plant-derived essential oils in nanoscale emulsions, these formulations enhance the solubility, stability, and bioavailability of active phytochemicals, leading to potent larvicidal, pupicidal, adulticidal, and insect growth regulatory effects. HNEs offer advantages such as rapid knockdown, low toxicity to non-target organisms, biodegradability, and reduced risk of resistance development. Despite these benefits, several research gaps remain, particularly in formulation standardization, long-term toxicity assessments, field-scale validation, and

regulatory approvals. The integration of nanotechnology with botanical insecticides offers a new frontier in vector management, but it requires a multidisciplinary approach involving entomology, toxicology, environmental science, and nanotechnology.

Future studies should focus on developing controlled-release nanoformulations, elucidating molecular mechanisms of action, and establishing field-level protocols for sustainable deployment. With continued research and regulatory support, HNEs have the potential to be incorporated into integrated vector management (IVM) programs as effective, safe, and environmentally compatible tools to combat mosquito-borne diseases such as dengue, malaria, and chikungunya.

Conflict of Interest

The authors declare no conflict of interest. The research and preparation of this review article were conducted independently, without any commercial, financial, or personal relationships that could be construed as a potential conflict of interest.

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