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A Review on the Use of Essential Oil-Based Nanoformulations in Control of Mosquitoes

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Abstract: It is estimated that one million deaths per year are caused by mosquito-borne diseases worldwide. While preventing such diseases is possible and, of course, more manageable than attended to treat patients. Prevention of these diseases is based on improving the environment (e.g., decreased stagnant water) and controlling mosquitoes in immature and adult forms. Resistances among mosquitoes, environmental pollution, and adverse effects on non-target species, such as humans, are some of the major disadvantages of using chemical insecticides. Essential oils (EO)s with a wide range of activities on mosquitoes, including ovicide effect, larvicide effect, pupicide effect, adulticide effects, and repellent effect, are proper alternatives for synthetic ones. However, their practical usage is questioned due to their volatility and lower efficiency than synthetic samples. In recent years, researchers have attended to overcome these challenges by formulating EOs into nanoformulations. In this study, existing reports on exploiting EO-based nanoformulations in mosquito control have been categorized as larvicides, repellents, and adulticides. Moreover, by discussing the reported results, the appropriate nanoformulations for each purpose have been suggested; polymeric nanoparticles are more suitable for larvicides, lipid nanocarriers are more suitable for repellents nanoemulsions are more suitable for adulticide.

Keywords: essential oil; nanoemulsion; polymeric nanoparticles; lipidic nanocarriers.

1. Introduction

Mosquitoes are the most important arthropods in public health [1,2]. They could transmit many diseases, such as malaria, dengue, yellow fever, chikungunya, encephalitis, and filariasis [3,4]. Malaria is the most important mosquito-borne disease; 228 million malaria cases and 405,000 deaths were reported worldwide just in 2018 [5,6]. Besides, the global incidence of *Aedes*-borne disease has grown dramatically in recent decades [7,8]. Nearly half the world's population is at risk of infection by dengue; ~ 400 million infections occur per year, of which approximately one-quarter of these patients manifest clinically [9].

Management of mosquitoes is a serious concern in developing countries facing vector-borne disease outbreaks [10,11]. Synthetic (chemical) insecticides are commonly used compounds for the control of both immature (egg, larva, and pupa) and mature stages of

mosquitoes [12,13]. Adverse effects on the environment (contaminating soil, water, and air), side effects on non-target populations (especially humans), and the development of resistance has increased considerably in recent years [14,15]. Furthermore, synthetic repellents' application to exposed skin to protect from mosquito bites is a common approach for reducing the transmission of mosquito-borne diseases and irritating bites [16]. However, there are concerns about their toxicity and safety [17,18]. These limitations have necessitated researchers to develop new compounds to combating mosquitos and preventing mosquito bites.

Essential oils (EO)s are naturally oily liquids that commonly extracted through hydro distillation from different parts (bark, stem, flower, and rhizome) of aromatic plants using the Clevenger type apparatus [19,20]. They have possessed a wide range of biological activities such as antibacterial effect [21,22], leishmanicidal effects [23,24], larvicidal effect [25,26], and repellent effects [27,28]. Recently, EO-based insecticides were introduced as alternatives to synthetic ones to control mosquitos because of their selective action on target and minimal side effects on non-target organisms and their high degradation in the environment [29,30]. The literature includes many studies about using EOs against mosquitoes [31,32]. However, applications of EOs as insecticide and repellent are limited because some of their ingredients are volatile.

Nanotechnology is targeted manipulations of materials in the nanoscale (especially 1 – 200 nm) for obtaining size-dependent features or functions [33,34]. The novel approach to stabilize EOs and improve their stability is formulating them into nanoformulations [35,36]. It has been accepted that by decreasing the droplets' size and increasing the surface-to-volume ratio, the solubility of EOs is improved, which ultimately leads to enhanced efficacy [37,38]. Formulating EOs into nanoformulation has recently been considered a promising approach for improving the stability and efficacy of EOs [39,40].

2. Review Methodology

Almost all reports (till 07.31.2020) on using only EO-based nanoformulations to control mosquitoes have been reviewed. For this purpose, three different channels, including the search engine (Google Scholar), indexed databases of scientific publications (PubMed), and also academic networks (Research Gate) were searched to find any original or review articles, commentaries, and reports related to this subject. Collected documents have been categorized as their reported approach to mosquito control, including larvicide, repellent, and adulticide. No research has yet been reported on the ovicidal and pupicidal effect of EO-based nanoformulations.

Due to the lipophilic nature of EOs, three types of formulations have been widely used for the preparation of EO-based nanoformulations; nanoemulsions, lipid-based nanocarriers (liposomes, solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC)), and polymeric nanoparticles. For a better discussion, a brief about the mentioned nanoformulations has been first given.

2.1. Nanoemulsions.

The mixture of two phases of water and oil is called emulsion; if droplet size is at the nanoscale, they called nanoemulsion [41]. Emulsions are classified as two basic categories of oil-in-water (O/W) and water-in-oil (W/O); in the former, oil as droplets are dispersed in water, but in the latter, vice versa (see Figure 1A and 1B). In both, surfactants or surface tension

reducing agents are used to mix the two phases. In particular, O/W nanoemulsions are much more frequently used because most of the drugs and all EOs are lipophilic and should be solubilized in blood flow or water (41, 42).

Nanoemulsions generally are prepared using high- and low-energy techniques. Ultrasonic-, a high-pressure homogenizer-, and microfluidizer-assisted fabrications belong to high energy methods. Low-energy techniques include phase inversion temperature, phase inversion composition, and solvent diffusion or spontaneous emulsification [42,43]. Given that some of the components of EOs are volatile, spontaneous emulsification is preferred over other approaches to prepare EO-based nanoemulsions. In this technique, nanoemulsions are prepared based on optimizing oil, water, and surfactant [35,44]. Thus, the prepared nanoemulsions in such a manner are not affected by physical and chemical stress, such as temperature and pH [45,46].

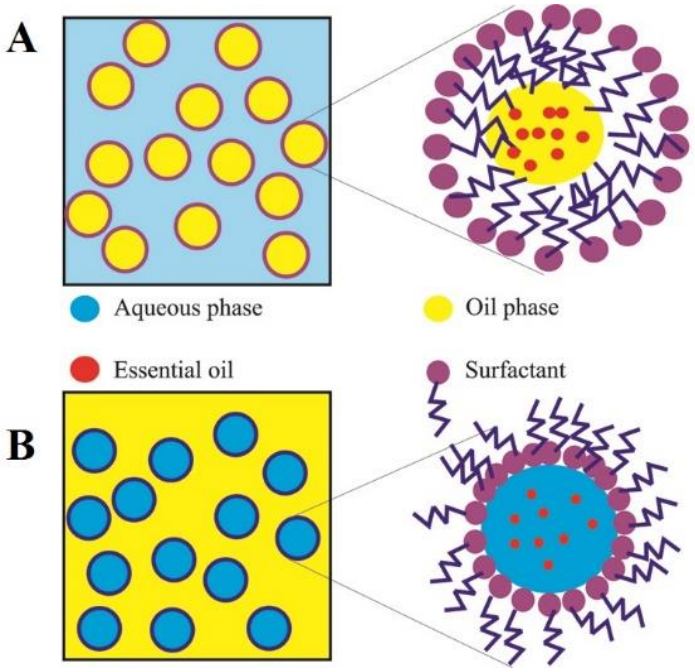

Figure 1. A: oil in water nanoemulsion, B: water in oil nanoemulsion.

2.2. Lipid-based nanocarriers.

Lipid-based carriers are suitable candidates for delivering various water-insoluble drugs and EOs due to the hydrophobic nature. They could increase drug molecules' solubility and stability, which led to improved pharmacokinetics' performance [47]. Moreover, compared to polymeric nanoparticles, their biocompatibility, and capacity to encapsulate highly lipophilic active substances are higher [48]. The following paragraphs describe some of the common lipid-based nanoformulations.

Liposomes composed of phospholipids and cholesterol; they formed a bilayered spherical structure by hydration in an aqueous medium (see Figure 2A). The presence of cholesterol in the liposome structure increases bilayers' stability and decreases cargos leakage [49]. Interestingly, both hydrophilic/phobic drugs could be loaded into liposomes; the phospholipid bilayer is a suitable space for hydrophobic drugs and the aqueous cavity for hydrophilic ones. Therefore, liposomes are commonly used in the pharmaceutical, cosmetic, and food industries [49]. Liposomes could increase the stability, solubility, and bioavailability of the drugs. Moreover, by encapsulating EOs into liposomes, they are preserved from oxidation and evaporation [50].

Depend on preparation methods, different liposomes from a few nanometers to micrometers have been reported. The common approaches are thin-film hydration, freeze-drying, reverse evaporation, and ethanol injection [51]. Among these approaches, thin-film hydration is mostly employed in the preparation of liposomes. In this method, lipid components containing a drug are dissolved in an organic solvent. The solvent is evaporated using rotary evaporation, and the lipid film is rehydrated in an aqueous solvent. Some techniques, including homogenization, sonication, freeze-thawing, and membrane extrusion, are used to control liposomes' size and size distribution [52].

Solid Lipid Nanoparticles (SLN) are biodegradable and biocompatible nanoformulation. They are prepared by dispersing physiological lipids and surfactants (for stabilization) in an aqueous phase in a size of 50 to 200 nm (see Figure 2B) [51,53]. In SLNs, the drug is dissolved in lipids with high melting points (> 40 ^{o}C) [54]. Since SLNs maintain they are solid-state at body temperature, they are attractive as long-acting or controlled-release formulations [47]. From the literature, SLNs commonly have been used for the delivery of different drugs, e.g., budesonide-loaded SLN for management of asthma [55], curcumin loaded SLNs to the improvement of oral bioavailability [56], SLN loaded kiteplatin for treatment of glioblastoma multiform [57]. Besides, SLNs have the potential to encapsulate EOs to control release. For example, Zhao et al. have prepared EO-loaded SLNs through a high-shearing homogenization technique for sustained inhalation [58].

Nanostructured Lipid Carriers (NLC) are another class of lipid nanoparticles that both liquid lipids solid lipids are used in their structure (see Figure 2C). Thus, their melting point is lower than SLNs; however, they preserved solid-state at room temperature. NLC is stabilized in an aqueous phase using a or combination of surfactants [59]. Many techniques for the preparation of NLC have been reported, e.g., displacement of solvents, micro emulsification, and high-pressure homogenizer. The high-pressure homogenizer is favorable, as no solvents are required in the preparation. Briefly, the mixture of drug-containing lipids is melted (about 10 °C above the melting temperature). The hot surfactant solution is then added and mixed using a high-pressure homogenizer [60].

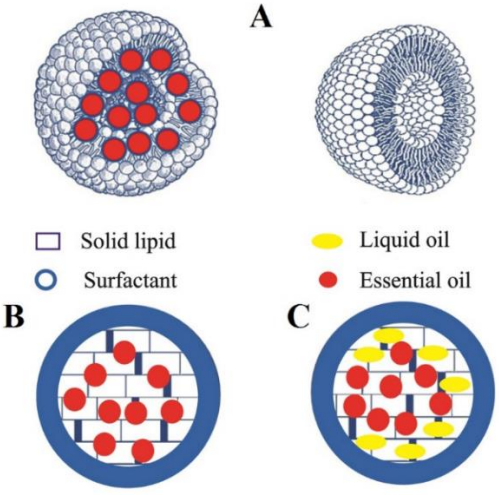

Figure 2. Lipid-based nanocarriers; A: liposome, B: solid lipid nanoparticles; C: nanostructured lipid carriers.

2.3. Polymeric nanoparticles.

Polymeric nanoparticles could be described as colloidal polymeric particles in a size range of 1–200 nm [61]. Polymeric nanoparticles could be biodegradable or non-biodegradable; however, the degradation rate and drug release rate could be modified using a

different mixture of polymers [37]. Some of the advantages of polymeric nanoparticles as drug/EOs carriers include proper control of the size, prolonged elimination, increased therapeutic effectiveness, simple preparation procedure, low toxicity, and drug preservation [62,63]. As demonstrated in Figure 3 A and B, cargoes could be incorporated in the matrix of polymeric nanoparticles (called nanospheres) or loaded in the core (called nanocapsules) [64,65].

Polymeric nanoparticles could be prepared using natural polymers such as chitosan, hyaluronic acid, and albumin, or from semi/synthetic polymers such as poly (lactide-co-glycolide) (PLGA), polyglycolic acid (PGA), polyacrylic acid (PAA), polylactic acid (PLA), and cellulose derivatives including carboxymethyl cellulose (CMC) and Hydroxypropyl methylcellulose (HPMC) [66,67]. Furthermore, nanoparticles are prepared using various methods, such as nanoprecipitation, solvent evaporation, ionotropic gelation, electrospray, salting out, and supercritical fluid technology [68,69].

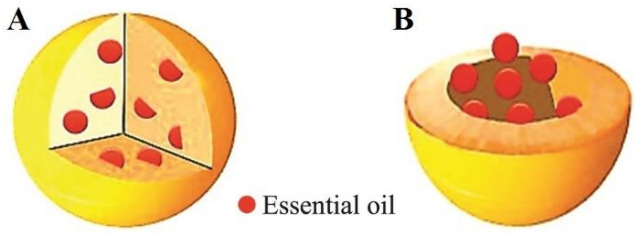

Figure 3. Polymeric nanocarriers: A: polymeric nanosphere, B: polymeric nanocapsule.

3. The Use of EO-based Nanoformulations for Control of Mosquitoes

Collected documents have been analyzed, and the use of EO-base nanoformulations are categorized as larvicide, repellent, and adulticide.

3.1. EO-based nanoformulations as larvicides.

Twenty EO-based nanoformulations as larvicides are listed in Table 1. Seventeen of them are nanoemulsions, and three others are chitosan nanostructures, including nanoparticles and nanobeads. Reasons for excessive utilization of nanoemulsions compared to other nanoformulations are easier access to the constituents and more straightforward preparation methods.

In the five first reports, only the larvicidal activity of nanoformulations was reported; therefore, the effect of nanoformulation on the efficiency of EOs could not be evaluated [70-73]. In such reports, the efficiency of nanoformulations should be compared with non-formulated EOs.

According to the other fifteen reports, the achievement of nanoformulation of EOs as larvicide are summarized into four classifications. In two reports, nanoemulsions' effects were significantly improved than non-formulated EOs at an examined concentration [74,75]. In another study, the perfect larvicidal effect (100% mortality) was achieved at 4 hours instead of 24 hours, related to non-formulated EO [76]. In 10 reports, reported lethal concentration 50 (LC_{50}) of nanoemulsions were significantly better than those of non-formulated EOs. In the two latest reports, continuity of larvicidal activity was investigated. Tarragon EO was encapsulated in chitosan nanoparticles at two different concentrations. The larvicidal effect of tarragon EO at concentrations of 1.6 and 6% was continued 2 and 4 days, while these times in nanoformulated forms were significantly improved, i.e., 4 and 9 days [77,78].

The physical stability of nanoemulsion is generally high; however, when they diluted during larvicidal tests (100-200 times), their stability is great decreases. Instability ultimately leads to short-term durability of the larvicidal effects [74,75]. Thus, the long-lasting larvicidal activity for nanoemulsions has not been reported; as described above, only the efficiency of EOs was improved.

The stability and loading capacity of lipidic nanocarriers for EOs is good. Besides, their spontaneous aggregation in the aqueous phase has been solved by exploiting different stabilization methods such as steric and electrostatic manners [79,80]. However, lipidic nanocarriers' preparation for spraying the environment on a large scale is not economically viable. In this regard, polymer nanoparticles (such as chitosan) with high stability and lower cost are preferred to prepare larvicides [77,78].

Furthermore, numerous articles have been published on the larvicidal properties of plant-synthesized silver nanoparticles. In such a manner, herbal extracts are used as reducing agents for the synthesis of silver nanoparticles from their salts, such as $AgNO_3$ or AgCl [81]. This method does not require toxic reducing agents [82,83]. Nevertheless, an important point has been overlooked in these reports; silver nanoparticles easily interact with chemical functional groups. Thus, silver nanoparticles' final properties are strongly dependent on reducing agent, i.e., herbal extracts [84,85]. For instance, reported LC_{50} of plant-synthesized silver nanoparticles was varied from 2 to 12470 ppm against *An. stephensi* [86,87]. In our previous study, chemically synthesized nanoparticles with a particle size of 30 nm showed only a 20% larvicidal effect at 100 ppm [88]. In general, silver nanoparticles are not good candidates for larvicidal purposes on a large scale due to their high price, environmental pollution, and varied efficacy.

Table 1. EO-based nanoformulations as larvicides.

References	EO names	Nanoformulation (size)	Target larvae	Achievement
[70]	*Ocimum basilicum*	nanoemulsion (28 nm)	*Cx. quinquefasciatus*	Larvicidal effect of nanoemulsion at 50 µg/mL: 100% EO no reported
[71]	*Azadirachta indica*	nanoemulsion (31.03)	*Cx. quinquefasciatus*	LC_{50} of nanoemulsion reported at 11.75 µg/mL EO no reported
[72]	*Pterodon emarginatus*	nanoemulsion (125 nm)	*Ae. aegypti*	Larvicidal effect of nanoemulsion at 250 µg/mL: 100% EO no reported
[73]	*Lippia alba*	nanoemulsion (117.0 nm)	*Ae. aegypti*	LC_{50} of nanoemulsion reported at 31.02 µg/mL EO no reported
[73]	*Lippia alba*	nanoemulsion (117.0 nm)	*Cx. quinquefasciatus*	LC_{50} of nanoemulsion reported at 38.22 µg/mL EO no reported
[74]	*Anethum graveolens*	nanoemulsion (10.7 nm)	*An. stephensi*	Larvicidal effect of the EO increased from 73.4 to 88.1 % at 60 µg/mL
[75]	*Artemisia dracunculus*	nanoemulsion (14.5)	*An. stephensi*	Larvicidal effect of the EO increased from 83.4 to 92.71% at 18 µg/mL
[76]	*Eucalyptus globulus*	nanoemulsion (9.4 nm)	*Cx. quinquefasciatus*	Larvicidal effect of EO at 250 µg/mL: 100% in 24 h exposure For nanoemulsion: 4 h exposure
[89]	*Citrus sinensis*	nanoemulsion (78.8 nm)	*Cx. pipiens*	LC_{50} of the EO decreased from 86.3 to 27.4 µg/mL
[90]	*Mentha piperita*	nanoemulsion (34 nm)	*Cx. pipiens*	LC_{50} of the EO decreased from 88.90 to 31.24 µg/mL
[91]	*Croton linearis*	nanoemulsion (163 nm)	*Ae. aegypti*	LC_{50} of the EO decreased from 64.24 to 17.86 µg/mL
[92]	*Anacardium occidentale*	nanoemulsion (52 nm)	*An. culicifacies*	LC_{50} of the EO decreased from 18.1 to 1.4 µg/mL

References	EO names	Nanoformulation (size)	Target larvae	Achievement
[93]	*Siparuna guianensis*	nanoemulsion (176 nm)	*Aedes aegypti*	LC_{50} of the EO decreased from 86.52 to 24.75 µg/mL
[94]	*Ricinus communis*	nanoemulsion (114 nm)	*An. culicifacies*	LC_{50} of the EO decreased from 52.3 to 3.4 µg/mL
[95]	*Ficus glomerata*	nanocrystal emulsion (104 nm)	*Ae. aegypti*	LC_{50} of the EO decreased from 60 to 20 µg/mL
[95]	*Ficus glomerata*	nanocrystal emulsion (104 nm)	*Cx. quinquefasciatus*	LC_{50} of the EO decreased from 48 to 22 µg/mL
[95]	*Ficus glomerata*	nanocrystal emulsion (104 nm)	*An. stephensi*	LC_{50} of the EO decreased from 60 to 17 µg/mL
[96]	*Eucalyptus globulus*	chitosan beads (200 nm)	*Cx. pipiens*	LC_{50} of the EO decreased from 20.301 to 0.419 µg/mL
[77]	*Artemisia dracunculus*	chitosan nanoparticles containing 1.6% EO (168 nm)	*An. stephensi*	Continuity of larvicidal effect of the EO increased from 2 to 4 days
[78]	*Artemisia dracunculus*	chitosan nanoparticles containing 6% EO (222 nm)	*An. stephensi*	Continuity of larvicidal effect of the EO increased from 4 to 9 days

3.2. EO-based nanoformulations as repellents.

One of the most synthetic insect repellents (without insecticidal effect) is DEET (N, N-diethyl-meta-toluamide), which is used as a gold standard in many repellency tests [97]. However, DEET's application has been questioned due to side effects such as allergy, dermatitis, cardiovascular and neurological disorders, and damage to the synthetic fabric and plastic [98,99]. So recently, much attention has been paid to the development of green repellents. Details of EO-based nanoemulsions as repellent are given in Table 2. Noted, microencapsulated lemongrass EO as repellent was also reported in the literature; however, that was related to preparing texture (polyester) with repellent activity [100].

A control group (chemical repellent or non-formulated EO) has not been reported in the two first reports in Table 2, so it is impossible to determine the advantages of using the nanoformulations. However, reported protection times are acceptable [101,102]; according to the Environmental Protection Agency (EPA), the minimum repellent time to obtain registration is 2 hours [103].

In the other reports, EO-based nanoformulations' efficiencies were comparable with synthetic repellents. For instance, protection times of nanoemulsion of *Eucalyptus globulus* (15%) and DEET 15% against a mixture of mosquitoes were reported at 170 and 211 min [104]. Besides, nanoemulsion of EOs of *Mentha piperita* (50%) and *Eucalyptus globulus* (50%) was prepared with protection times of 257 and 351 min against *An. stephensi*; this time for DEET 25% was 370 min [105].

Furthermore, the protection time of nanocrystal emulsion of *Ficus glomerata* was comparable with an Odomos (synthetic repellent) against three mosquitoes [95]. For the preparation of this formulation (FON), a nanoemulsion of neem oil (NON) was first prepared. Then, ethanol extract of the plant was added for crystallization of emulsion. The repellent effects of FON, NON, and Odomos were reported as follows; *Ae. aegypti*: 234, 192, and 223 min, *An. stephensi*: 238, 198, and 230 min, and *Cx. Quinquefasciatus*: 233, 193, and 229 min [95].

The stratum corneum cells (corneocytes) are dense, functionally dead, anucleated, and filled with keratin. Also, some lipids are forming several bilayers surrounded the corneocytes. Intercellular fat consists of a mixture of ceramides, cholesterol, and fatty acids [106]. Reviewing the literature, a substance with moderate lipophilicity could transit through the

stratum corneum, but the more hydrophilic substance is inhibited in the epidermis and dermis. However, when skin hydrated, the stratum corneum considerably swells and shows increased permeability [79,97].

Given that nanoemulsions contained a high amount of water; therefore, part of the formulation penetrates the skin after hydration. The other part evaporates. It is also topically usage of nanoemulsions are challenges due to low viscosity. Therefore, contrary to existing reports, nanoemulsions are not a suitable formulation as repellents.

Furthermore, high lipophilic materials accumulate in the skin's uppermost layers, where their action as sunscreen or repellent should occur [79]. Incorporating sunscreen (or repellent) in the lipidic nanocarriers appeared to bind to keratin for an extended period and serve as a reservoir for elongation of their activity [80]. Therefore, lipidic nanocarriers with higher viscosity (easier topical usage) are better candidates for preparing EO-based nanoformulations as repellents.

Table 2. EO-based nanoformulations as repellents.

References	EO names	Nanoformulation (size)	Target mosquitoes	Protection time
[101]	*Cymbopogon nardus*	nanoemulsion (135 nm)	*Ae. aegypti*	168 min No control
[102]	*citronella (10%) hairy basil (5%) vetiver (5%)*	nanoemulsion (153.7)	*Ae. aegypti*	282 No control
[104]	*Eucalyptus globulus*	nanoemulsion (17.1 nm)	*Cx. pipiens (62%), Ochlerotatus caspius (22%), Cx. pusillus (10%) Cx. tritaeniorhynchus (6%).*	170 min DEET: 211 min
[105]	*Mentha piperita*	nanoemulsion (11.32 nm)	*An. stephensi*	257 min DEET: 370 min
[105]	*Eucalyptus globulus*	nanoemulsion (103.90 nm)	*An. stephensi*	351 min DEET: 370 min
[95]	*Ficus glomerata*	nanocrystal emulsion (104 nm)	*Cx. quinquefasciatus*	233 min Odomos: 229 min
[95]	*Ficus glomerata*	nanocrystal emulsion (104 nm)	*An. stephensi*	238 min Odomos:230 min
[95]	*Ficus glomerata*	nanocrystal emulsion (104 nm)	*Ae. aegypti*	234 min Odomos:223 min

3.3. EO-based nanoformulations as adulticides.

Only one document has been found on the adulticide effect of EO-based nanoformulations. Nanoemulsion of *Ocimum sanctum* EO was prepared; however, its particle size was not reported. The knockdown effect (KD_{50}) of the nanoformulation after one-hour exposure with *Ae. aegypti* and *Cx. quinquefasciatus* were reported as 7.01 and 4.05 mg/cm^2. Its lethal dose fifty (LD_{50}) after 24 hours of exposure were also reported at 28.60 and 20.09 mg/cm^2 [107].

However, not many studies have been reported in this area (EO-based nanoformulations as adulticides). In residual spraying, the toxin's effect should not remain on the wall, so it seems that nanoemulsions are proper candidates for this purpose. Most of their contents are water, and it is also possible to prepare them on a large scale.

4. Conclusions

According to existing studies and available sciences, polymeric nanoparticles are more suitable for larvicides, lipid nanocarriers are more suitable for repellents, and nanoemulsions

are more suitable for adulticide. Moreover, some recommendations worth to be mentioned: a) performing bioassay tests under field conditions, b) test of side effects of EO-based nanoformulations on non-target organisms, c) determination of the insecticidal effect of EO-based nanoformulations against other medically important vectors, d) attempt to the application of EO-based nanoformulations in the vector control industry.

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Conflicts of Interest

The authors declare no conflict of interest.

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