



The Role of Plant Essential Oils in Mosquito (Diptera: Culicidae) Control

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Authors' contributions

This work was carried out in collaboration between all authors. Author MEAN conducted the literature searches, wrote the first draft and effected the reviewers' corrections. Author NIEW produced the families of plant species yielding essential oils. Author REO classified the major constituents of terpenoids. Author SNO conceived the title and wrote the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Approximately half of the world's population is at risk of malaria, and about 250 million deaths are reported annually. There are two strategic approaches to malaria management: prevention (vector control, drug prophylaxis and potential use of vaccines) and treatment (drugs, blood transfusion, etc). The use of botanicals, specifically essential oils is an alternative to the current use of synthetics and pyrethroids. Essential oils can be used as larvicides and repellents. The major constituents of those that have been used in mosquitoes are mainly terpenoids (Acyclic Monoterpenoids, Monocyclic Monoterpenoids, Sesquiterpenoids, Bicyclic Monoterpenoids, Diterpenoid). Earlier, *in vitro* physicochemical assays characterized most of them as antioxidants. However, recent studies suggest that at least in part, the encountered beneficial effects of essential oils are due to pro-oxidant effects at the cellular level. Approximately 40 plant species in about 10

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families dominated by Labiateae (Lamiaceae) and Asteraceae, have provided essential oils for experimental studies as larvicides and repellents to mosquitoes. Results from both studies have been very promising. LC_{50} s varied across plant and mosquito species and across geographical locations within plant species. In repellency tests, RD_{50} also varied across plant and mosquito species. Some of these essential oils are promising candidates as larvicides in Integrated Vector Management (IVM) in Malaria Management that combines: Indoor Residual Spraying (IRS), use of Insecticide-Treated Net (ITN) and larviciding. As repellents, they will contribute to the reduction in human-mosquito contact, an important component of protection, a strategic approach to malaria management.

Keywords: Essential oils; terpenoids; labiateae; control; mosquitoes.

1. INTRODUCTION

Currently, the risk of contracting arthropod-borne diseases has increased due to climate change and globalization [1]. Malaria, a life-threatening disease transmitted by mosquitoes is still a major health problem, affecting children and adults around the world, especially in tropical countries. About 3.3 billion people, half of the world's population is at risk of the disease. Annually, 250 million cases and nearly one million deaths are reported [2]. Four subgenera of *Anopheles* are involved in the transmission of human malaria parasites across 12 Epidemiologic zones globally [2].

There are two main strategic approaches for malaria management:

(a) Malaria prevention (Vector Control, Drug prophylaxis and potential use of vaccine) and (b) treatment (drugs, blood transfusion, among others) [3]. Due to the absence of an operational vaccine, parasite resistance to anti-malarial drugs, and resistance of malaria transmitting mosquitoes to insecticides, alternative methods for disease control are vital [4]. Two forms of vector control, IRS (Indoor Residual Spraying) and ITN (Insecticide-Treated Net) are generally applicable for reducing disease transmission [3]. The use of insecticide-treated nets (ITNs), especially pyrethroid-based has been widely promoted, not because they are more powerful but because they are simpler and potentially less demanding than IRS [5]. However, they are potentially more vulnerable to insecticide resistance as only pyrethroid insecticides are recommended for use on ITNs, owing to toxicity and efficacy concerns [6]. IRS is the application of stable formulations of insecticides to the inside of houses to kill resting adult female mosquitoes. The primary contribution of IRS

in reducing malaria transmission is: reducing the life span of female mosquitoes so that they can no longer transmit malaria parasites, and reducing the density of vectors [7]. Although formulations have improved, most IRS formulations last less than 4 months [3]. Currently, the main classes of insecticides used for vector control are organophosphates, carbamates, organochlorines and pyrethroids.

Efforts are being made to expand the number of classes. An initiative, the Innovative Vector Control Consortium (IVCC), a Product Development Partnership, aims to reduce peridomestic pathogens through improved vector control with innovative products. Several studies have identified the range and function of olfactory receptors in the mosquito, with the long-term aiming to develop new attractants and repellents that could play an operational role in vector control [8-9]. An Integrated approach to Vector Management (IVM), comprising IRS, ITN and larviciding, has been recommended by the World Bank [10].

2. PLANT-DERIVED INSECTICIDES (BOTANICALS)

Using plant derivatives or botanical insecticides is one of the alternatives. Their use in agriculture dates back to ancient times. Isman [11] enumerated four major types of botanical products used for insect control (Pyrethrum, Rotenone, Neem, Essential oils), along with three others in limited use (Ryania, Nicotine, Sabadilla). While botanicals are now a small part of the overall pesticide market, due to replacement by synthetics, the new environmental movement has provided a favourable environment for the re-birth of botanical insecticides. Public resistance to adoption of Genetically Modified Organisms (GMOs) is another factor favouring alternative

control measures such as the use of biopesticides [11]. Botanicals have certain advantages and probably an equal number of disadvantages. The advantages of botanical insecticides lie in their rapid degradation and lack of persistence and bioaccumulation in the environment, which have been the major problems in synthetics. The diversity of phytochemicals in botanical extracts is also useful. Redundancy is the presence of numerous analogues of one compound and is known to increase efficacy of extractives in metabolism of compounds and prevent the evolution of insecticide resistance when selection occurs over several generations [12]. Despite these advantages, there are major challenges: economical supply of plants products, quality control, lack of stability and costly toxicology testing for new products which may have limited Intellectual Property (IP) Protection and a relatively small market [12].

3. PLANT ESSENTIAL OILS

Plant essential oils are produced commercially from several botanical sources, many of which are of the mint family, Labiateae (Lamiaceae) [11]. The oils are generally composed of complex mixtures of monoterpenes biogenetically related phenols and sesquiterpenes [11]. A number of the sources from plants have been traditionally used for protection of stored commodities, especially in the Mediterranean region and in Southern Asia, but interest in the oils was renewed with emerging demonstration of their fumigant and insecticidal activities to a wide range of pests in the 1990s [13]. Certain plant oils, widely used as fragrances and flavours in the perfume and food industries, have long been reputed to repel insects. Investigations in the past 3-4 decades in several countries confirm that some plant oils not only repel insects but have contact and fumigant insecticidal actions against specific pests, and fungicide actions against important plant pathogens [13]. Essential oils possess a wide spectrum of biological activities including anti-microbial, fungicidal, insecticidal, insect repellent, herbicidal, acaricidal, and nematicidal. Since the middle ages, essential oils have been widely used for bactericidal, insecticidal, fungicidal, anti-parasiticidal, medicinal and cosmetic applications especially in pharmaceutical, sanitary, cosmetic, agricultural and food industries. Since the mode of extraction is mostly by distillation from aromatic plants, they contain a variety of volatile molecules such as terpenes and terpenoids,

phenol-derived aromatic compounds. The rapid action against some pests is indicative of a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine [14-15] by some oils and with GABA-gated chloride channels by others [16]. *In vitro* physicochemical assays characterize most of them as antioxidants [17]. However, recent studies show that in eukaryotic cells, essential oils can act as pro-oxidants affecting inner cell membranes and organelles such as mitochondria [17]. Depending on the type and concentration, they exhibit cytotoxic effects on living cells but are usually non-genotoxic. In some cases, changes in intracellular redox potential and mitochondrial dysfunction induced by essential oils can be associated with their capacity to exert anti-genotoxic effects. These findings suggest that at least in part, the encountered beneficial effects of essential oils are due to pro-oxidant effects at the cellular level [17].

4. SOURCES AND CONSTITUENTS OF ESSENTIAL OILS IN MOSQUITOES

The major constituents of essential oils used in mosquito control, either as larvicides or repellents were: linalool, Geranial, β -Citronella, Eucalyptol, Perilladehyde, Grandisol, Cuminaldehyde, Limonene, Carvone, Piperifenone oxide, Carvacrol, Thymol, Terpinen-4-ol, P-Cymene, α -Pinene, Camphene, α -Thujone, Camphor, Fenchone, α -Clemene, α -Gurjunene, Patchoulol, Allylbenzenes (Phenylpropenes), Eugenol, Methyl eugenol, Methyl Chavicol (Estragiol), trans-Anethol, Asaricin and 16-Kaurene [18-34]. These compounds can be classified as either terpenes or generally terpenoids (which include hydrocarbons and other derivatives). Classification of terpenoids is based on the number of isoprene units (C_5H_8) (Table 1) [35]. These essential oils occur in about 10 families, dominated by Labiateae (Lamiaceae) and Asteraceae [36-45] (Table 2).

5. EFFECTS OF ESSENTIAL OILS ON MOSQUITOES

Plant essential oils have been used on different species of mosquitoes, including *Culex pipiens*, *Culex quinquefasciatus*, *Anopheles arabiensis*, *Anopheles gambiae* s.s., *Anopheles gambiae* s.l., *Anopheles braziliensis*, *Anopheles dirus*, *Anopheles cracens*, *Aedes aegypti* and *Aedes albopictus*. They have been used as arvicides [18,19,24-27,29-33,46-50], repellents [4,18,20,

33,48,51-54], adulticides [55] and ovicides [56]. Essential oils have also been shown to be anti-plasmodial [30,57]. As larvicides, the LC_{50s}, after 24 hrs varied across plant and mosquito species. On *Aedes aegypti*, the LC_{50s} after 24 hrs, varied from 140.2 mg/L (Rutaceae) [33] to 28.4-56.7 µg/ml (Cupressaceae) [27], 54 µg/ml (Piperaceae-*P. hostmaninum*) [47], 156 µg/ml (Piperaceae- *P. humatanum*) [47] and 36 µg/ml (*P. permucronatum*) [47]. On *Aedes albopictus*, the LC_{50s}, after 24 hrs, varied from 40.8-144.4 µg/ml (Lauraceae) [25], to 51.2-57.9 µg/ml (Cupressaceae) [27]. The LC₅₀, after 24 hrs, on *Anopheles stephensi* was 12.8 ppm (Asteraceae) [21]. On *Culex pipiens*, the LD_{50s}, after 24hrs, varied from 61.25 µg/L (Labiatae-*Melissa officinalis*) [29] to 78.28 µg/L (Labiatae- *Mentha longifolia*) [29], 57.85 µg/L (Labiatae- *Mentha spicata*) [29], 91.45 µg/L (Labiatae- *Salvia fructosa*) [29] and 79.46 µg/L (Labiatae- *Salvia pamifera*) [29]. In *Culex quinquefasciatus*, the LC_{50s}, after 24 hrs, varied from 43.6 µg/ml (Labiatae- *Thymus saturoides*) to 32.9 µg/ml (Labiatae-*Thymus vulgaris*) [58].

Table 1. Classification of terpenoids

Monoterpenoids (C₁₀H₁₆ and compounds)	
Acyclic Monoterpenoids	
Linalool	3,7-Dimethyl-1,6-octadien-3-ol
Geranial	3,7-Dimethyl-2,6-octadienal
β-Citronella	Mixture of (Citronellol) 3,7-Dimethyl-6-octen-1-ol and Geranial
Monocyclic Monoterpenoids	
Eucalyptol (same as 1,8-Cineol)	1,8-Epoxy-p-menthane
Perillaldehyde	4-Mentha-1,8-dien-7-al
Grandisol	Cis-2-isopropenyl-1-methylcyclobutane ethanol
Cuminaldehyde	4-isopropylbenzaldehyde
Limonene (a precursor to carvone)	4-Isopropenyl-1-methylcyclohexene (p-Mentha-1,8-diene)
Carvone	5-Isopropenyl-2-methyl-2-cyclohexenone
Piperitenone oxide (Carvone oxide)	Carvone oxide
Carvacrol (isomeric with thymol)	5-Isopropyl-2-methylphenol
Thymol	2-Isopropyl-5-methylphenol
Terpinen-4-ol	4-Hydroxy-4-isopropyl-1-methylcyclohexene
p-Cymene	4-Isopropyltoluene (an alkylbenzene related to monoterpane)
Bicyclic Monoterpenoids	
α-Pinene	Bicyclomonoterpene
Camphene	Bicyclomonoterpene
α-Thujone	4-Methyl-1-isopropylbicyclo[3.1.0]hexan-3-one
Camphor	Bicyclomonoterpene (has a keto group)
Fenchone (norbornanone)	Bicyclomonoterpene (has a keto group)
Sesquiterpenoids (C₁₅H₂₄ and compounds)	
α-Elemene	
α-Gurjunene	
Patchoulol	
Viridiflorol	
Caryophyllene oxide	
Eugenol	4-Allyl-2-methoxyphenol (5-Allyl-2-hydroxyanisole)
Methyl eugenol	4-Allyl-1,2-dimethoxybenzene
Methylchavicol (Estragol)	4-Allylanisole
trans- Anethol	p-Propenylanisole [1-Methoxy-4-(1-propenyl)benzene]
Asaricin	5-Methoxy-6-(2-propenyl)-1,3-benzodioxide
Allylbenzenes (Phenylpropenes) – can also be considered as monoterpenoids because of the number of isoprene units – they are C ₁₀ H ₁₆ compounds.	
Diterpenoids (C₂₀H₃₂ and compounds)	
16-Kaurene	

Table 2. Families of plants whose essential oils were used on mosquitoes

s/n	Scientific names	Families	Common names
1	<i>Achillea millefolium</i> Linn.	Asteraceae	Yarrow, tea plant
2	<i>Cinnamomum verum</i> JS Presl	Lauraceae	Cinnamon tree
3	<i>Clausena anisata</i> (Wild.) Hook. f.	Rutaceae	Clausena
4	<i>Conyza sumatrensis</i> (Retz.) E.H. Walker	Asteraceae	Horse weed, fleabane
5	<i>Cryptomeria japonica</i> (Thunb ex L.F.) D. Don.	Cupressaceae	Red wood, Cypress
6	<i>Cymbopogon citratus</i> (DC) Stapf.	Poaceae	Lemon grass
7	<i>Dendropanax morbifera</i> Leveille	Araliaceae	Ginseng
8	<i>Eucalyptus camalduensis</i> Mehn	Myrtaceae	Gum plant
9	<i>Eugenia uniflora</i> Linn.	Myrtaceae	Suriname cherry
10	<i>Foeniculum</i> sp. Mill	Apiaceae	Sweet fennel
11	<i>Helichrysum foetidum</i> Linn. Moench	Asteraceae	Stinking straw flower, everlasting flower, licorice plant
12	<i>Lavandula coronifolia</i> Poir.	Labiataeae	English lavender
13	<i>Lippia multiflora</i> Moldenke	Verbenaceae	Scented matgrass, tea bush
14	<i>Melisa officinalis</i> Linn	Labiataeae	Common balm
15	<i>Mentha</i> sp. Linn.	Labiataeae	Mint plant
16	<i>Myrthus</i> sp. Linn.	Myrtaceae	Myrtle
17	<i>Ocimum basilicum</i> Linn.	Labiataeae	Sweet basil, mint plant
18	<i>Piper guineensis</i> Schum. & Thonn.	Piperaceae	Black pepper
19	<i>Plectranthus assurgens</i> (Bak.) JK Morton	Labiataeae	Mexican mint
20	<i>Rosemarinus officinalis</i> Linn.	Labiataeae	Rosemary plant
21	<i>Salvia splendens</i> Ker-Grawl	Labiataeae	Kitchen sage
22	<i>Satureja robusta</i> (Hook. f) Brenan	Labiataeae	Summer savory
23	<i>Tagetes patulus</i> Linn.	Asteraceae	Aztec marigold, African marigold
24	<i>Tarchonanthus camphoratus</i> Linn.	Asteraceae	Camphor plant
25	<i>Tetradlea</i> indet	Asteraceae	
26	<i>Teucrium polium</i> Linn.	Labiataeae	Wood sage, American germander
27	<i>Thymus vulgaris</i> Linn.	Labiataeae	Thyme
28	<i>Carapa procera</i> Dc.	Meliaceae	Kind Oil tree
29	<i>Khaya senegalensis</i> (Desr) A. Juss.	Meliaceae	Mahogany
30	<i>Sesamum indicum</i> Linn.	Pedaliaceae	Beniseed
31	<i>Duranta repens</i> Linn.	Verbenaceae	Mosquito plant/
32	<i>Croton macrostachyus</i>	Euphorbiaceae	Golden Drewberg
33	<i>Ipomoea cairica</i> (L.) Sweet	Convolvulaceae	Mile-a-minute/coastal morning glory
34	<i>Momordica charantia</i> Linn.	Cucurbitaceae	Bitter melon
35	<i>Tridax procumbens</i> L.	Asteraceae	Coat buttons
36	<i>Lantana camara</i> Linn.	Verbenaceae	Wild sage
37	<i>Hyptis suaveolens</i> (L.) Poit.	Labiataeae	Spikenard
38	<i>Litsea cubeba</i> Linn.	Lauraceae	Exotic Verbena, Tropical verbena, Chinese pepper
39	<i>Melaleuca leucadendron</i> Linn.	Myrtaceae	Cajuput tree, Fine leaf melaleuca, Punk tree, Swamp tea tree, White tea tree, White wood
40	<i>Nepeta cataria</i> L.	Labiataeae	Catnip, Catswort, Catmint

Mentha oil was most promising against *Anopheles stephensi* and *Aedes aegypti*, recording LC₅₀ values of 39.74 and 46.23 ppm respectively for larvicidal activity. Calamus oil was the most effective against *Culex quinquefasciatus* with LC₅₀ value of 40.40 ppm

for larvicidal activity [49]. The repellent activity of *Lantana camara* using the method of "separated arm" was evaluated against *Anopheles stephensi*. The LD₅₀, after 24 hrs, was 61 ppm [56]. Essential oils from five plant species (*Piper sarmentosum*, *Foeniculum vulgare*, *Curcuma*

longa, *Myristica fragrans*, *Zanthoxylum piperitum*) were evaluated against *Anopheles cracens* [59]. The strongest larvicidal potential was from *P. sarmentosum*, followed by *F. vulgare*, *C. longa*, *M. fragrans* and *Z. piperitum* with LD₅₀ values of 16.03, 32.77, 33.61, 40.00 and 63.17 ppm respectively. Binary mixtures between *P. sarmentosum* and the others at the highest ratio proved to be highly efficacious with cototoxicity coefficient value greater than 100, indicating synergistic activity [59].

As repellents, the RD₅₀ ($\times 10^{-5}$) of essential oils to *Anopheles gambiae* varied from 8.9/cm² (Asteraceae) to 26 mg/cm² (Verbenaceae- *Lippia javonica*), 8.9/cm² (Labiaceae), 240 mg/cm² (Asteraceae- *Tarhonathus camphoratus*), 50 mg/cm² (Asteraceae- *Tarhonathus riparia*) [20]. In repellency tests with three major mosquito vectors, *Anopheles stephensi* (Liston), *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* (Say), the five most effective oils were Litsea (*Litsea cubeba*), Cajeput (*Malaleuca quinquenervia*), Violet (*Viola odorata*) and Catnip (*Nepeta cataria*), which induced a protection time of 8h at the maximum and 100% repellency against these vectors [60]. The 1%v/v solution and 15%v/w cream and ointment of lemongrass, *Cytopogon* oil, exhibited $\geq 50\%$ repellency, lasting 2-3 h against *Aedes aegypti*, which may be attributed to citral, a major constituent. This activity was comparable to that of a commercial mosquito repellent. Base properties of lemongrass oil formulation influenced their effectiveness [51]. The essential oils of *Ipomoea cairica*, *Momordia charantia* and *Tridax procumbens* exhibited high repellency effect (>300 minutes at 6% concentration) against *Anopheles stephensi*, followed by *Contella asiatica* and *Psidium guajava*, which showed less effect (<150 minutes at 6% concentration). However, the ethanol-applied arm served as control and provided 8.0 minutes repellency [33]. Volatile oils from *Croton pseudopulchellus* Pax., *Mkilua fragrans* Verde (Annonaceae) *Endostemon tereticaulis* (Poir) Ashby, *Ocimum forskolei* Benth, *Ocimum fischeri* Guerke and *Plectranthus longipes* Baker (Labiataeae) from the Kenyan coast were evaluated for repellency on forearms of human volunteers against *Anopheles gambiae* s.s. All oils were found to be more effective RC₅₀ range-0.67-9.21 $\times 10^{-5}$ mg/cm² than DEET [52] (RC₅₀ – 33 $\times 10^{-5}$ mg/cm²). Essential oil of *Conyza newii* Oliv. & Hiern (Asteraceae) from northern Kenya was highly repellent to *Anopheles gambiae* s.s. (RD₅₀ = 8.9 $\times 10^{-5}$ mg/cm²). RC₅₀ was the concentration

that repelled 50% of the population, while RD₅₀ was the dose that repelled 50% of the population. Furthermore, when seedlings of *C. newii* were propagated in seven different geographical regions of Kenya, they exhibited significant variations in the relative proportions of the constituents and this was reflected in differences in repellency of the essential oils [61]. Concentrations of 10% or 50% essential oils from *Cytopogon nardus* (Citronnella), *Pogostemon cablin* (Patchuli), *Syzygium aromaticum* (Clove) and *Zanthoxylum limonella* (Makaen) used against *Culex quinquefasciatus*, *Anopheles dirus* and *Aedes aegypti* did not prevent mosquito bites for as long as 2 h, but the undiluted oils were the most effective and provided 2 h complete repellency. Clove oil gave the longest duration of 100% repellency (2-4 h) against all species [62]. DEET, at concentrations of 20% or more showed the best efficacy, providing to 10h protection against *Aedes aegypti*. Citriodora (plant-derived) repellency against *Aedes aegypti* was lower. Differences occurred within genera. *Aedes aegypti* proved more difficult to repel than *Aedes albopictus*. Results with *Anopheles gambiae* were similar. *Culex quinquefasciatus* was easier to repel. Many plant-based repellents provide short duration protection. Adding 5% vanillin to plant-based and DEET repellents increased protection by about 2 h. The differences in LC₅₀ and LD₅₀ values may be partly associated with variations in experimental environmental conditions [54].

Essential oils have also shown ovicidal and adulticidal activities. The LD₅₀ of *Lantana camara* oil on eggs was 53.59 ppm. *Hyptis suaveolens* and *Ocimum camum* exhibited repellent activities against adult mosquitoes. The most effective was *H. suaveolens* with a 50% efficacy at a concentration level of 67 ppm. The results suggest that those essential oils have a potential for vector control and can be considered as a source of natural and environmental-friendly substances for malaria vector control [56].

6. CONCLUSION

Using plant derivatives (botanicals) is one of the alternatives to persistent, non-target, very toxic synthetics. Essential oils are a group of botanicals. In addition to the general advantages of botanicals, redundancy, presence of numerous analogues of one compound, which is known to increase efficacy by synergism and slows the onset of insecticide resistance is a characteristic of essential oils. The major

constituents are terpenoids in those that have been used as larvicides or repellents against mosquitoes. Sources are from several plant species across more than 10 families dominated by the Labiateae (Lamiaceae) and Asteraceae. They have been found effective as larvicides. Repellency tests have shown these oils as providing protection for several hours, and in some cases comparable to the protection from synthetic DEET. Essential oils are therefore useful in larviciding, an approach advocated by the World Bank, to complement Indoor Residual Spraying (IRS) and the use of an Insecticide Treated Net in an Integrated Vector Management (IVM). As repellents, essential oils reduce human-mosquito contact, ensuring protection, one of the two strategic approaches to malaria control.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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