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*Review Article*

# Plant Volatile Oils and Compounds as Ecofriendly Mosquito Control Products: Review on Recent Developments

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**ABSTRACT:** Mosquitoes occupy the first place among the most dangerous insects in the world, because they kill more than five lakhs people every year around the world by transmitting lethal pathogens. People use many methods and devices to combat the mosquito problem. Chemical pesticides are commonly used to control mosquitoes and their bites around the world. Since the last two decades public has started to avoid the use of synthetic chemicals for mosquito management due to their harmful effects on environment and human health. Pesticide resistance in mosquitoes is a major side effect of synthetic chemical applications. Volatile oils or essential oils obtained from green plants are considered as reliable alternatives for mosquito management. Several hundred volatile oils and volatile compounds have been tested against eggs, larva, pupa and adults of various vector mosquito species. Oils, extracts and powders of leaf, flowers and bark of many aromatic plants are being used as mosquito repellents in many countries for many centuries. Scientific validation of biological activities of several botanicals has been intensified in the last three decades and several herbal mosquito control products have been commercialized. The aim of the present review article is to provide up-to-date information on biological activities of plant volatile oils and volatile compounds against vector mosquitoes and to highlight the promising volatile compounds for the development of new herbal mosquito control products.

**Keywords:** volatile oils, 1,8- cineole, thymol, larvicides, repellents, mosquitoes.

## INTRODUCTION

Mosquitoes are major public health pests throughout the world. They are distributed all over the world, except Antarctica. World mosquito fauna comprises 3541 species grouped under 112 genera (Tyagi et al., 2015); more than a hundred species of mosquitoes are capable of transmitting various diseases to humans and other vertebrates. Female mosquitoes transmit many pathogenic organisms and cause diseases such as malaria, West Nile virus (WN), dengue, filariasis, yellow fever, Japanese encephalitis and Chikungunya among humans. Mosquito bites also cause considerable pain and loss of sleep; weight loss and decreased milk production in farm animals have been reported due to mosquito bites (Nour et al., 2009).

At present several synthetic insecticides and growth regulators namely methoprene, pyriproxyfen and diflubenzuron and microbial products from *Bacillus thuringiensis israelensis* and *B. sphaericus* are used in mosquito control programmes (Shaalan et al., 2005; Ben-Dov, 2014; Lacey et al., 2015). Pyrethroids and malathion are recommended as adulticides and temephos (an organophosphate insecticide better known as Abate®), petroleum oils, S-methoprene (Insect growth regulator), *Bacillus thuringiensis israelensis* and *B. sphaericus* are recommended as mosquito larvicides (Brattsten et al., 2009). Vector mosquitoes have developed resistance against many synthetic pesticides due to injudicious use of pesticides and in fact this is the major reason for the resurgence of many mosquito-borne diseases (Becker et al., 2003). Since 1947 more than 100 mosquito species have been reported as resistant to one or more insecticides (Hemingway and Ranson, 2000; Hemingway *et al.*, 2002).

Prevention of mosquito bite is the most common approach among the public. Personal protection by means of avoidance techniques such as using physical and chemical barriers, treatment of fabric with toxicants and the use of topical (skin) repellents is the commonly advocated approach for preventing mosquito bite (Barnard and Xue, 2004).

Plant products are considered as ecofriendly insecticides and are potential alternatives to synthetic insecticides. Pyrethrum, derris, quassia, nicotine, hellebore, anabasine, azadirachtin, d-limonene camphor and turpentine were widely used as alternate insecticides in developed countries before the introduction of synthetic organic insecticides (Wood, 2003). Plant essential oils are ecofriendly ‘green pesticides’ and safe to mammals and environment (Mossa, 2016). Essential oils are complex mixtures of compounds mainly of monoterpenes and sesquiterpenes. Several scientific studies have disclosed the efficacy of plant volatile oils and volatile compounds in pest and vector management (Mondal and Khalequzzaman, 2006; Paulraj and Ignacimuthu, 2007; Pavunraj et al., 2007; Sivanandhan et al., 2021). Many volatile oils have larvicidal, pupicidal and oviposition deterrent effects against vector mosquitoes (Chantawee and Soonwera, 2018)

The present review article highlights the promising volatile oils and plant volatile compounds which show significant larvicidal and repellent activities against common vector mosquitoes.

## LARVICIDAL ACTIVITY OF VOLATILE OILS AND VOLATILE PLANT COMPOUNDS

Mosquito control programmes are largely targeting the larval stage in their breeding sites with larvicides (El Hag et al., 1999 & 2001). Larviciding is a successful method of reducing mosquito population in their breeding places before they emerge into adults (Tiwary et al., 2007). Application of synthetic larvicides in aquatic ecosystems may cause harmful effects on human beings and many other non-target organisms. In search of alternative pesticides many investigators have explored the beneficial role of plant extracts, plant compounds, actinomycetes sea weeds and mushroom extracts against mosquito larvae (Campbell and Sullivan, 1993; Thangam and Kathiresan, 1994; Pandey et al., 2007; Nazar et al., 2009; Ganesan et al., 2018; Darvin et al., 2019; Sivanandhan et al., 2019; Monisha et al. 2020). Many essential oils showed good larvicidal activities against mosquito species and are reported as environmentally safe insecticides (Corbet et al., 1995; Pitasawat et al., 2007). Some effective essential oils as larvicides against different mosquito species are given in Table 1.

**Table 1.** Some effective volatile oils and their major components reported for mosquito larvicidal activity

| Essential oil source | Target species | Effective concentration or $LC_{50}$ | Major components in the oil | Reference |
|---|---|---|---|---|
| *Boswellia serrata* | *Culex pipiens* | 83.36 ppm | NS | Khater and Shalaby (2007) |
| *Brassica compestris* | | 71.37 ppm | | |
| *Calocedrus formosana* Florin Bark | *Aedes aegypti* | 51.8 mg/ml | NS | Cheng et al. (2003) |
| *C. formosana* Florin leaf | | 56.3 mg/ml | NS | |
| *Carum ptroselinum* | *Cx. pipiens* | 152.94 ppm | NS | Khater and Shalaby (2007) |
| *Chenopodium ambrosioides* (aerial parts) | *Anopheles arabiensis* | 17.5 ppm | | Massebo et al. (2009) |
| | *A. aegypti* | 9.1 ppm | | |
| *Cinnamomum camphora* leaves | *Anopheles stephensi* | LC50 = 0.026%<br>LC95 = 0.128% at 24 h | Eucalyptol (53.49%)<br>B-terpinene (17.44%)<br>α-terpineol (9.45%) | Xu et al. (2020) |

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|---|---|---|---|---|
| *Citrus aurantium* subsp. *Bergamia* fruit rind (cold pressed) | *Cx. pipiens* | 58.73 mg/L (48 h) | Linalyl acetate | Eleni et al. (2009) |
| *C. aurantium* subsp. *Bergamia* fruit rind (hydrodistillation) | | 106.6 mg/L (48 h) | Linalool and Limonene | |
| *C. aurantium* subsp. *Bergamia* Leaves | | 68.53 mg/L (48 h) | Linalyl acetate and Limonene | |
| *Corymbia citrodora* leaves | *An. arabiensis* | 40.3 ppm | | Massebo et al. (2009) |
| | *Ae. aegypti* | 38.7 ppm | | |
| *Cryptomeria japonica* | *Ae. aegypti* | 48.1 mg/ml | NS | Cheng et al. (2003) |
| *C. japonica* leaf | | 37.6 mg/ml | NS | |
| *Curcuma aromatica* | *Ae. aegypti* | 36.3 ppm | | Choochote et al. (2005) |
| *Cymbopogon citrates* Stapf. | *Ae. aegypti* | 69 ppm (24 h) | geranial (60.3%) and neral (39.7%). | Cavalcanti et al. (2004) |
| *C. nardus* (L.) Rendle (2nd Fraction) | *Cx. quinquefasciatus* | 1.7 mg/l (1.4 mg/l after 3 months of storage at 29°C) | Myrcene | Ranaweera and Dayananda (1996) |
| *Cymbopogon winterianus* (Citronella oil) | *Ae. aegypti* | LC50= 111.84 µg $mL^{-1}$ | NS | Cansian et al. (2021) |
| *Cyperus esculentus* | *Cx. pipiens* | 47.17 ppm | NS | Khater and Shalaby (2007) |
| *Dendropanax morbifera* flowers | *Ae. aegypti* | 62.32 ppm | *γ*-elemene (18.59%), tetramethyltricyclohydrocarbon (10.82%), *β*-selinene (10.41%), *α*-zingibirene (10.52%), 2-isopropyl-5-methylbicylodecen (4.2%), *β*-cubebene (4.19), and 2,6-bis(1,1-Dimethylethyl)-4-phenol (4.01%). | Chung et al. (2009) |
| *Eruca sativa* | *Cx. pipiens* | 86.06 ppm | NS | Khater and Shalaby (2007) |
| *Eucalyptus montana* leaves | *An. arabiensis* | 68.3 ppm | | Massebo et al. (2009) |
| | *Ae. aegypti* | 52.9 ppm | | |
| *Ipomoea cairica* Linn. | *Cx. tritaeniorhynchus* | 14.8 ppm | - | Thomas et al. (2004) |
| | *Ae. aegypti* | 22.3 ppm | | |
| | *An. stephensi* | 14.9 ppm | | |
| | *Cx. quinquefasciatus* | 58.9 ppm | | |
| *Lavandula angustifolia* | *Cx. pipiens* | LC50 = 140µg/ml LC=90=450µg/ml | Linalool (32.23%) Linalyl acetate (14.23%) | El-Akhal et al. (2021) |
| *Lavandula dentata* | | LC50=2670 µg/ml LC90=7400 µg/ml | 1,8-Cineole (49.82%) Camphor (6.31%) | |
| *Lippia adoensis* leaves | *An. arabiensis* | 56.4 ppm | | Massebo et al. (2009) |
| | *A. aegypti* | 47.1 ppm | | |
| *L. sidoides* | *Ae. aegypti* | | Thymol | Carvalho et al. (2003) |
| *L. sidoides* | *Ae. aegypti* | 63 ppm (24 h) | Thymol (80.8%); p-Cymene (8.6%); Trans-Caryophyllene (5.1%) | Cavalcanti et al. (2004) |
| *Mentha spicata* leaves | *An. arabiensis* | 85.9 ppm | NS | Massebo et al. (2009) |
| | *Ae. aegypti* | 67.8 ppm | | |
| *Nigella sativa* leaves | *An. arabiensis* | 23.4 ppm | NS | |
| | *Ae. aegypti* | 32.1 ppm | | |

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|---|---|---|---|---|
| *Ocimum americanum* L. | *Ae. aegypti* | 67 ppm (24 h) | E-Methyl-Cinnamate (70.9%); Z-Methyl-Cinnamate (8.8); Trans-Bergamoptene (6.8%); Trans-Caryophyllene (4.6%) | Cavalcanti et al. (2004) |
| *O. basilicum* L (four accessions) | *Anopheles* sp. | 190 µL/L to 300 µL/L | eugenol, geraniol and linalool | Nour et al. (2009) |
| *O. gratissimum* L. | *Ae. aegypti* | 60 ppm (24 h) | Eugenol (43.7%); 1,8-cineole (32.7%); Z-Ocimene (6.2%); Trans-Caryophyllene (4.1%) | Cavalcanti et al. (2004) |
| *O. lamiifolium leaves* | *An. arabiensis* | 20.9 ppm | | Massebo et al. (2009) |
| | *Ae. aegypti* | 8.6 ppm | | |
| *O. suave* leaves | *An. arabiensis* | 53.5 ppm | | Massebo et al. (2009) |
| | *Ae. aegypti* | 29.8 ppm | | |
| *Pinus longifolia* (pine oil) | *An. stephensi* | 112.6 ppm | K–terpineole (12.89%); Isoeugenol (4.93%); Eugenol (3.14%); Caryophyllene (2.94%) | Ansari et al. (2005) |
| | *Ae. aegypti* | 82.1 ppm | | |
| | *Cx. quinquefasciatus* | 85.7 ppm | | |
| *P. gaudichaudianum* | *Ae. aegypti* | 121 µg/ml | Viridiflorol (27.5%), Aromadendrene (15.55 %), β-Selinene (10.5%), Ishwarane (10%) | Morais et al. (2007) |
| *P. hostmanianum* | | 54 µg/ml | Asaricin (27.37%), Myristicin (20.26%), Dillapiol (7.66%), Germacrene D (6.82%), Piperitone (5.58%) | |
| *P. humaytanum* | | 156 µg/ml | Caryophyllene oxide (16.63%), β-Selinene (15.77%), Spathulenol (6.33%), b-Oplopenone (6.02%), Sesquicineole (5.03%) | |
| *Piper marginatum* Jacq. (leaves) | *Ae. aegypti* | 23.8 ppm | (Z)-Asarone (30.4%), Patchouli alcohol (16%), Elemol (9.7%), Bicyclogermacrene (9.4%), (E)-Caryophyllene (7.5%), (E)-Asarone (6.4%) | Autran et al. (2009) |
| *P. marginatum* (stem) | | 19.9 ppm | (E)-Asarone (32.6%), Patchouli alcohol (25.7%), (Z)-Asarone (8.5%), Elemicin (6.9%), (E)-Caryophyllene (6.8%), Seychellene (5.8%), (E)-Methyl isoeugenol (3.6%) | |
| *P. marginatum* (inflorescence) | | 19.9 ppm | Patchouli alcohol (23.4%), (E)-Asarone (22.1%), (E)-Caryophyllene (13.1%), α-Acoradiene (9.7%), α-Copaene (9.4%), (Z)-Asarone (4.5%), δ-Elemene (3.1%) | |
| *P. nigrum* seeds | *An. arabiensis* | 33.5 ppm | NS | Massebo et al. (2009) |
| | *Ae. aegypti* | 9.1 ppm | | |
| *P. permucronatum* | *Ae. aegypti* | 36 µg/ml | Dillapiol (54.7%), Myristicin (25.61%), Elemicin (9.92%), Asaricin (8.55%) | Morais et al. (2007) |

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|---|---|---|---|---|
| *Satureja montana* (stems, leaves and flowers) | *Cx. pipiens* biotype *molestus* | 37.7 mg/L | Carvacrol (55.42%), γ-terpinene (13.24%), *p*-cymene (9.45%) | Michaelakis et al. (2007) |
| *S. thymbra* (stems and leaves) | | 44.5 mg/L | Thymol (42.15%), γ-terpinene (20.12%), *p*-cymene (10.39%) | |
| *S. thymbra* (stems, leaves and flowers) | | 64.4 mg/L | Carvacrol (30.39%), Thymol (24.32%), γ-terpinene (14.64%), *p*-cymene (9.19%) | |
| *S. spinosa* (stems and leaves) | | 56.1 mg/L | Carvacrol (47.12%), Thymol (12.39%), γ-terpinene (6.49%), *p*-cymene (5.48%), *β*-caryophyllene (4.98%), thymol, methyl ether (4.05%) | |
| *S. parnassica* ssp. *parnassica* (stems and leaves) | | 52.1 mg/L | Thymol (44.39%), γ-terpinene (12.32%), *p*-cymene (8.35%), Carvacrol (6.36%), *β*-caryophyllene (4.42%) | |
| *Schinus molle* leaves | *An. arabiensis* | 21.0 ppm | NS | Massebo et al. (2009) |
| | *Ae. aegypti* | 9.6 ppm | | |
| *S. molle* seeds | *An. arabiensis* | 26.5 ppm | NS | |
| | *Ae. aegypti* | 14.5 ppm | | |
| *Thymus vulgaris* leaves | *An. arabiensis* | 33.7 ppm | NS | |
| | *Ae. aegypti* | 17.3 ppm | | |
| Thymus vulgaris | *Aedes aegypti* | --- | Thymol | Maia et al. (2019) |
| *Trigonella foenumgrecum* | *Cx. pipiens* | 32.42 ppm | NS | Khater and Shalaby (2007) |
| *Zanthoxylum armatum* DC seeds | *Cx. quinquefasciatus* | 49 ppm | Linalool, Limonene, E-methyl cinnamate | Tiwary et al. (2007) |
| | *Ae. aegypti* | 54 ppm | | |
| | *An. stephensi* | 58 ppm | | |
| *Zingiber cassumunar* | *Ae. albopictus* | LC50=44.9 µg/L in 24 h | (-) terpinen-4-ol (1) (isolated by bioassay-guided isolation) | Li et al. (2021) |
| *Ziziphora clinopodioides* Lam. | *An. stephensi* | 14.9 (µg/ml) | Pulegone, Piperitone, Menth-2-en-1-ol and carvacrol | Mohammadreza (2008) |
| | *Cx. pipiens* | 16.5 (µg/ml) | | |

NS- Not Studied

Larvicidal activity of essential oils of nine Brazilian plants was studied against *Aedes aegypti*. *Ocimum americanum* and *O. gratissimum* oils recorded 100 per cent mortality at 100 ppm concentration and their effect was comparable to *Lippia sidoides* and *Cymbopogon citrates* (Cavalcanti et al., 2004). Plant volatile oils contain a range of bioactive compounds. Identification and confirmation of larvicidal components present in the active essential oils by separation techniques such as TLC and GC-MS is an essential part of work in the bioassay experiments. Nour et al. (2009) have documented the larvicidal activity of essential oils obtained from four Sudanese accessions of Basil (*Ocimum basilicum*) against *Anopheles* larvae. They found that accession no. 16 (introduced from UA Emirates) and 17 (introduced from Germany) were more effective with $LC_{50}$ of 190 and 200 µL $L^{-1}$ respectively. They reported that eugenol, geraniol and linalool were the major volatile components in the basil essential oils and these compounds caused 100 per cent larval mortality within half an hour of exposure. Essential oil of *Tridax procumbens* leaves showed larvicidal activity against *Aedes aegypti* at $LC_{50}$ concentration of 79.0 $\mu$g mL$^{-1}$ and 69.15 $\mu$g mL$^{-1}$ in 24 and 48 h, respectively. Thymol (48.22%), *γ*-Terpinene (15.93%), *o*-Cymene (10.27%) and Carvacrol methyl ether (7.7%) were detected as major compounds in *T. procumbens* essential oil by GC-MS analysis method (Brandão et al., 2021). Essential oils of 28 plant species were tested individually against *Cx. quinquefasciatus* larvae and adults and binary combinations of effective oils were again tested. Combination of *Allium sativum* (bulbs) and *Citrus paradisi* (leaves) oils at 1:1 volume ratio recorded the highest larvicidal activity and GC-MS analysis revealed the presence of diallyl disulfide, linalool, citronellal and caryophyllene oxide as major constituents (Mahanta and Khanikor, 2021).

It is generally concluded that the major component present in the volatile oil may be the actual cause of larval death. To confirm this, the individual compound of interest should be tested for its activity. Some studies have indicated that the volatile compounds thymol and carvacrol were responsible for larvicidal activity against mosquito larvae. Carvalho et al. (2003) studied the larvicidal activity of essential oil of *Lippia sidoides* and its major components thymol and carvacrol against *Ae. aegypti*. They reported that the pure essential oil and dilution of oil at 1: 2 ratio recorded 100 per cent larval mortality within 5 minutes and thymol recorded 100 per cent larval mortality at 0.04% within 30 min. Dilutions of oil at 1: 5 and 1: 10 ratios caused 100 per cent larval mortality within 20 minutes and after 24 h respectively. Carvacrol did not cause any larval mortality at 0.04% concentration. Cavalcanti et al. (2004) have also reported the larvicidal activity of *L. sidoides* oil against *Ae. aegypti*. Their study clearly indicated that 63 ppm of *L. sidoides* oil killed 50 per cent larvae in 24 h and they reported that the oil contained thymol (80.8%) as the major component. Besides *L. sidoides* oil Cavalcanti et al. (2004) screened eight more essential oils from Brazilian plants against *Ae. Aegypti* larvae. They found that the essential oils of *Ocimum americanum*, *O. gratissimun* and *C. citratus* were as potent as *L. sidoides* and caused 100 per cent mortality at a concentration of 100 ppm. Michaelakis *et al*. (2007) analysed the essential oils of *Satureja montana* and *S. thymbra*, which showed high toxicity against the larvae of *Culex pipiens* biotype *molestus* and found that carvacrol or thymol were the major components. According to them the larvicidal activity was a result of the synergistic effect of the phenolic compounds with other components that were present in the oils tested. In a study essential oils were extracted from leaves of 10 *Piper* spp. and larvicidal activity was studied against strains of Pyrethroid resistant and susceptible *Ae. Aegypti.* Among the ten species, five species viz., *Piper aduncum, P. marginatum, P. gaudichaudianum, P. crassinervium*, and *P. arboretum* were found to be active since they recorded larvicidal activity between 90–100% at 100 ppm concentration. The active compounds in the oils namely *(E)*-Anethole, β-Asarone, γ-Terpinene, *p*-Cymene, Limonene, α-Pinene, β-Pinene and Dillapiole showed larvicidal activity between 90-100% at 100 ppm (Filho et al., 2021). Knio et al. (2008) studied the larvicidal activity of essential oils extracted from commonly used medical and culinary herbs in Lebanon against the fourth instar larvae of seaside mosquito *Ochlerotatus caspius*. They found that thyme inflorescence extract was the most potent larvicide followed by parsley seed oil, aniseed oil and coriander fruit oil. Based on GC-MS analysis they reported that thymol, sabinene, carvacrol, anethole and linalool were the major components of parsley seeds and leaves, alpine thyme inflorescences, anis seeds and coriander fruits respectively. These studies support the fact that thymol in volatile oils is a toxicant against mosquito larvae. (Cavalcanti et al., 2004; Cheng et al., 2004; Choochote et al., 2005; Knio et al., 2008; Maia et al., 2019)

Tiwary et al. (2007) studied the larvicidal activity of volatile oils obtained from the seeds of *Zanthoxylum armatum* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*. They analysed the volatile oils by GC-MS and found that linalool (57%) and limonene (19.8%) were the major components. Linalool was reported as mosquito repellent by Omolo et al. (2004) and Traboulsi et al. (2005). At the same time

Traboulsi et al. (2002) and Cheng et al. (2004) discovered that linalool at higher doses caused larval mortality. The study by Chantraine et al. (1998) indicated that linalool did not cause larval mortality in *Ae. aegypti* at doses ranging between 10 and 100 ppm.

Kelm and Nair (1998) isolated two compounds from hexane extract of *Ocimum sanctum* leaves and stem and screened them against *Ae. aegyptii* larvae. They claimed that eugenol and (*E*)-6-hydroxy-4,6-dimethyl-3-heptene-2-one exerted larvicidal activity at 200 and 6.25 µg $mL^{-1}$ concentrations respectively in 24 h.

Several investigators have reported that 1,8-cineole is mainly responsible for pesticidal properties against many insects (Duke, 2004). Lucia et al. (2007) studied the larvicidal activity of *Eucalyptus grandis* and pine resin essential oils (turpentine) and their major components namely α- and β-pinene and 1,8-cineole against third and fourth instar *Ae. aegypti* larvae. They recorded that turpentine oil was more active ($LC_{50}$ of 14.7 ppm) than the essential oil of *E. grandis* ($LC_{50}$: 32.4 ppm), which contained 1,8-cineole. Klocke et al. (1987) found that 1,8-cineole, present in the volatile oil of *H. fitchii,* did not exhibit any significant mosquito larvicidal activity. But the oil acted as a moderate feeding repellent and highly effective ovipositional repellent against adult *Aedes aegypti.*

Chung et al. (2009) have reported that the essential oil obtained from *Dendropanax morbifera* flowers had a significant toxic effect against early fourth instar larvae of *Ae. aegypti* with an $LC_{50}$ and $LC_{90}$ of 62.32 and 131.21 ppm respectively and the oil contained 27 compounds; the major components were γ-elemene (18.59%), tetramethyl tricyclohydrocarbon (10.82%), β-selinene (10.41%), α-zingibirene (10.52%), 2-isopropyl-5-methylbicyclodecen (4.2%), β-cubebene (4.19%) and 2,6-bis (1,1-Dimethylethyl)-4-phenol (4.01%). The pure compound that was isolated from the essential oils gave higher larvicidal activity than crude essential oils. Tripathi et al. (2004) have reported that the compound piperitenone oxide that was isolated from oil of *Mentha spicata* variety *viridis* recorded higher larvicidal activity ($LD_{50}$=61.64 µg/ml) than the crude essential oil ($LD_{50}$=82.95 µg/ml) against *An. stephensi.*

Cheng et al. (2009) evaluated the larvicidal activity of leaf essential oils of six chemotypes of cinnamon (*Cinnamomum osmophloeum*) against *Ae. albopictus, Armigeres subalbatus* and *Cx. quinquefasciatus.* In their study the leaf essential oils of cinnamaldehyde type ($LC_{50}$=40.8 µg/ml; $LC_{90}$ = 81.7 µg/ml) and cinnamaldehyde/cinnamyl acetate type ($LC_{50}$=46.5 µg/ml; $LC_{90}$ = 83.3 µg/ml) recorded the maximum inhibitory effect against *Ae. albopictus* larvae. They also reported that the effective constituents in leaf essential oils were *trans*-cinnamaldehyde and benzaldehyde and the $LC_{50}$ values of these constituents against *A. albopictus* larvae were below 50 µg/ml.

The larvicidal activity of essential oils varies between different mosquito species. Pitasawat et al. (2007) have reported that among the aromatic oils obtained from five different plant species, *Carum carvi*, *Apium graveolens*, *Foeniculum vulgare*, *Zanthoxylum limonella* and *Curcuma zedoaria, Z. limonella* oil was the most effective against *A. aegypti* with $LC_{50}$ and $LC_{95}$ values of 24.61 and 55.81 ppm, respectively and *C. zedoaria* oil was the most effective against *A. dirus* larvae ($LC_{50}$ and $LC_{95}$ values: 29.69 and 40.23 ppm, respectively). Eleven essential oils out of 12 obtained from plants namely *Chenopodium ambrosioides* (aerial parts), *Ocimum lamiifolium* (leaves), *O. suave* (leaves), *Schinus molle* (leaves and seeds), *Piper nigrum* (seeds), *Corymbia citriodora* (leaves), *Eucalyptus globules* (leaves), *Lippia adoensis* (leaves), *Mentha spicata* (leaves) and *Thymus vulgaris* (leaves) were evaluated. They showed higher larval toxicity in *Ae. Aegypti* than *An. arabiensis* after 24 h exposure and oil obtained from *Nigella sativa* (leaves) recorded higher toxicity against *An. arabiensis* (Massebo et al., 2009). In a review Shaalan et al. (2005) had stated that mosquito larvae of different species displayed different susceptibilities to the same phytochemical and *Aedes* larvae, in general, were robust and less susceptible to insecticides and botanicals than *Culex* larvae.

## IMPROVING THE LARVICIDAL ACTIVITY OF VOLATILE OILS

Studies on the improvement of the larvicidal activity of volatile oils have been done by some investigators. Corbet et al. (1995) have studied the usefulness of some surfactants to increase the larvicidal activity of essential oils. In their study they used 1% insoluble surfactant (Arosurf MSF) and 1% detergent with eucalyptus and turpentine oils separately. They reported that refined turpentine at a dose of 2 µl per tub (=0.13 µl $cm^{-2}$) acted faster due to the addition of surfactants (turpentine: Arosurf: detergent 100:1:1 by volume) causing higher mortality in fourth instar *Cx. pipiens* form *molestus* at 24 and 48 h after treatment, than familiar surface-active larvicide Arosurf alone.

## REPELLENT PROPERTY OF VOLATILE OILS

The relationship between man and blood sucking insects is very old and man has learnt adequate techniques to avoid the mosquito biting. Keeping away the adult mosquitoes from human habitats and preventing mosquito bites are vital tactics in personal protection from mosquito borne diseases. Repellents are the prime products used for the protection from mosquito bites. Due to the enormous developments in science and technology new insect repellents are being discovered. Synthetic repellents are the most commonly used materials against mosquitoes. DEET (N, N-diethyl-3-methylbenzamide) is the most commonly used synthetic mosquito repellent. Insect repellent property of DEET was first discovered in 1953 and the first DEET product was introduced in 1956 (Peterson and Coats, 2001). It is an effective insect repellent against a broad spectrum of insects including mosquitoes.

Injudicious use of synthetic skin repellents which contain DEET may affect human health (Qui et al., 1998; Eden et al., 2020). Some investigators have pointed out the allergic reactions and human toxicity of DEET (Robbins and Cherniack, 1986; Edwards and Johnson, 1987; Qui et al., 1998). Peterson and Coats (2001) have noted that encephalopathy in children, urticaria syndrome, anaphylaxis, hypotension and decreased heart rate are some toxic effects associated with DEET. Trigg (1996), Walker et al. (1996), Debboun et al. (2000), Peterson and Coats (2001), Fradin and Day (2002) and Badolo et al.(2004) have pointed out that DEET has the ability to act as a good solvent for plastics and other synthetic materials and search for alternative repellents including natural products are essential. Since DEET is a chemical, a concentration of less than 10 per cent is recommended for children.

The novel repellents are mainly based on human safety. Electronic mosquito repellent is said to be environmentally friendly. But these electronic devices have been shown to have no effect as a mosquito repellent (Andrade and Bueno, 2001). In many countries aromatic plants are playing important role in mosquito management since ancient times. Very early report on mosquito repellent properties of essential oils was made by Penfold and Morrison (1952). They have reported the repellent and insecticidal activities of forty Australian essential oils against vector insects including mosquitoes. They reported that the essential oils obtained from *Dacrydium franklini, Backhousia myrtifolia, Mela-leuca bracteata* and *Zieria smithii* were the most effective.

In African countries *Hyptis suaveolens, Ocimum* spp. and *Daniellia oliveri* are used as traditional repellents against mosquitoes (Curtis et al., 1991). Aromatic plants contain volatile oils and volatile compounds which are responsible for their mosquito repellent property. In table 2 the mean protection time or repellency time of some essential oils against different mosquito species is given. The essential oil of *Ocimum suave* contained eugenol and six terpenoid substances and was an effective mosquito repellent (Chogo and Crank, 1981). Literature on the repellent activity of volatile oils against different mosquito species is plenty (Yang and Ma, 2005; Gillij et al., 2008). Repellency of volatile oils extracted from six plant species namely *Croton pseudopulchellus*, *Mkilua fragrans*. *Endostemon tereticaulis*, *Ocimum forskolei*, *O. fischeri* and *Plectranthus longipes* was evaluated against *An. gambiae sensu strict* (Odalo et al., 2005). Citronella and pennyroyal essential oils have been used as repellents against insects since ancient times (Jantan and Zaki, 1999).

**Table 2.** Repellent activity of some essential oils against vector mosquitoes

| Volatile oil source | Target mosquito | Repellency/ Protection time | Effective concentration | Reference |
|---|---|---|---|---|
| *Acantholippia seriphioides* | *Aedes aegypti* | 70 min | 50 % | Gillij et al. (2008) |
| *Aloysia citriodora* | | 90 min | 12.5 % | |
| *Baccharis spartioides* | | 90 min | 12.5% | |
| *Cinnamomum mollisimum* | *Ae. aegypti* | 100% | 0.0379 mg $cm^2$ | Jantan and Zaki (1999) |
| Citronella + vanillin | *Ae. aegypti, Anopheles dirus, Culex quinquefasciatus* | 100% (6 h), | 0.1 ml of 25% oil in 5% vanillin per 3x10 cm skin in the arm | Tawatsin et al. (2001) |
| *Citrus limon* Burm | *An. stephensi* | | 1% | Oshaghi et al. (2003) |
| *C. citronella* (lemon grass oil) | *An. culicifacies* | 100 % | Pure oil (1 ml) | Ansari et al. (2005) |
| | *Cx. quinquefasciatus* | 98.5% | | |
| *Cymbopogan martinii martinii* Stapf var *sofia* | *An. sundaicus* | 8 hrs (98.7%) (in indoor) and 6 hrs (96.52%) (in outdoor) | Pure oil (1ml) | Das and Ansari (2003) |
| *Cymbopogan nardus* | *Ae. aegypti* | 72.2% | 0.0047 mg $cm^2$ | Jantan and Zaki (1999) |
| *Eucalyptus saligna* | *Ae. aegypti* | 90 min | 50 % | Gillij et al. (2008) |
| *Litsea elliptica* | *Ae. aegypti* | 100% | 0.0379 mg $cm^2$ | Jantan and Zaki (1999) |
| *Melissa officinalis* L. | *An. stephensi* | | 1% | Oshaghi et al.(2003) |
| *Minthostachys mollis* | *Ae. aegypti* | 60 min | 50 % | Gillij et al. (2008) |
| *Nepeta parnassica* (vegetative stage) | *Cx.pipiens* | 88.5 % | 10 mg | Gkinis et al. (2003) |
| Pinus longifolia | *An. culicifacies* | 100 % | Pure oil (1 ml) | Ansari et al. (2005) |
| | *Cx. quinquefasciatus* | 97.4 % | | |
| Piper aduncum | Ae. aegypti | | | Misni et al. (2008) |
| *Pogostemon cablin* | *Ae. aegypti* | 71.4% | 0.0047 mg $cm^2$ | Jantan and Zaki (1999) |
| *Rosmarinus officinalis* | *Ae. aegypti* | 90 min | 50% | Gillij et al. (2008) |
| *Tagetes minuta* | | 90 min | 25 % | |
| *Zanthoxylum piperitum* + 5% vanillin | *Ae. aegypti* (In laboratory study) | 2.5 h* | 0.1 ml of oil in 3x10 cm skin area | Kamsuk et al. (2006) |
| | *Ae. gardnerii*, *An. barbirostris*, *Armigeres subalbatus*, *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Cx. vishnui* and *Mansonia uniformis* | 100% (for 120 min) (in field condition) | | |

*Median complete protection time

The mosquito repellent property of volatile oils is measured in terms of total protection time. *Piper aduncum* essential oil gave 95.2% protection against *Ae. albopictus* bites under laboratory conditions (Misni et al., 2009); the protection was reduced to 83.3% after 4 h, 64.5% after 6 h, and 51.6% after 8 h postapplication. Deet is used as standard reference in the repellent bioassay studies which involves plant volatile oils or volatile compounds. Oshaghi et al. (2003) compared the effectiveness of lemon and melissa oils with Deet and reported that lemon (92.70%) and melissa (92.67%) oils gave less protection than Deet (97%) against *An. stephansi* in guinea-pigs; however these differences were not significant. Tawatsin et al. (2001) extracted volatile oils from four plant species namely turmeric (*Curcuma longa*), kaffir lime (*Citrus hystrix*), citronella grass (*Cymbopogon winterianus*) and hairy basil (*Ocimum americanum*) by steam distillation method and evaluated the repellent activity against three mosquito vectors *Ae. aegypti*, *A. dirus* and *Cx. quinquefasciatus*. They found that the volatile oils from turmeric, citronella grass and hairy basil significantly gave maximum protection from three mosquito species and the repellency was increased for up to eight hours when 5% vanillin was added with the oils. They also reported that the standard repellent DEET gave protection for at least eight hours against only two mosquito species *Ae. aegypti* and *Cx. quinquefasciatus* but DEET with 5% vanillin gave protection against all the three mosquito species for at least eight hours.

Das and Ansari (2003) have reported that 1 ml of the essential oil of *Cymbopogan martinii martinii* var *sofia* oil when applied on the body parts gave 8 (98.7%) and 6 h (96.52%) of protection in indoor and outdoor respectively against *An. sundaicus* when tested for a period of 12 h. They reported that palmarosa oil contained geraniol (76.15%) as the major component.

Maguranyi et al. (2009) evaluated the repellency of essential oils from 11 Australian native plants against *Ae. aegypti, Cx. quinquefasciatus*, and *Cx. annulirostris* under laboratory conditions. Based on the preliminary results they selected the three most effective oils, mixed them and tested the repellency. *Prostanthera melissifolia* essential oil gave the longest protection time (110 min.) against *Cx. quinquefasciatus* and the repellency against *Ae. aegypti* was increased when the blend of *Leptospermum petersonii*, *Prostanthera melissifolia*, and *Melaleuca alternifolia* was tested at 5% v/v. They recommended the use of the blend of the above three oils as a short term repellent or under conditions of low mosquito abundance.

The effectiveness of mosquito repellents depends on many environmental factors. According to Barnard et al. (1998) the effect of repellents can vary greatly among mosquito species. A repellent will not protect all users equally and several factors such as species of the mosquito, density of organisms in the immediate surroundings, user's age, sex and biochemical attractiveness to the biting insect, ambient temperature, humidity and wind speed determine the effectiveness of a repellent (Golenda et al., 1999; Maibach et al., 1966; Muirhead-Thomson, 1951; Fradin, 2001; Fradin and Day, 2002). The essential oils obtained from different parts or during different stages of the same plant may have variations in their components, which is considered as a major cause for their differential activities. Gkins et al. (2003) isolated essential oils from the aerial parts of *Nepeta parnassica* at both vegetative and flowering stages and evaluated them against *Cx. pipiens molestus*. They recorded a significant repellent activity at 10 mg of oil obtained from the vegetative stage of *N. parnassica* when compared with the flowering stage, which was due to the qauantitative variation in the components of both oils. The main metabolites of vegetative-stage oil were recorded as 4a$\alpha$,7$\alpha$,7aβ-nepetalactone (22.0%), 1,8-cineole (21.1%), $\alpha$-pinene (9.5%) and 4a$\alpha$,7β,7aβ-nepetalactone (7.9%).

## ENHANCING THE REPELLENT ACTIVITY OF VOLATILE OILS

Most of the essential oils have a short protective duration against mosquitoes due to their high volatility (Kamsuk et al., 2006). This limitation can be overcome by making a formulation with some fixatives like liquid paraffin (Oyedele et al. 2002) vanillin (Tawatsin et al. 2001), salicyluric acid (Blackwell et al., 2003), and mustard and coconut oils (Das et al. 2003). In a field study it was observed that the essential oil of *Zanthoxylum piperitum* fruits with 5% vanillin provided better protection against a wide range of mosquito populations than oil without vanillin; vanillin-oil combination was also found to be more effective than 25% DEET (Kamsuk et al., 2006). Choochote et al. (2007) have reported the repellent property of essential oils of fruits, rhizomes and seeds of ten plants namely *Amomum xanthioides*, *Curcuma zedoaria*, *Kaempferia galanga*, *Anethum graveolens*, *Apium graveolens*, *Carum carvi*, *Foeniculum vulgare*, *Piper longum*, *Zanthoxylum limonella* and *Zanthoxylum piperitum* against *Ae. aegypti*. The complete protection time of individual oils and mixtures of two effective oils with and without 10 per cent vanillin was studied. *Z. piperitum*, *A. graveolens* and *K. galangal* provided repellency against *A. aegypti* with median complete-protection times of 1, 0.5 and 0.25 h, respectively and the protection time of *Z. piperitum*, *Z. limonella*, *K. galangal* and *C. zedoaria* increased significantly when 10% vanillin was incorporated. The highest median complete protection time (2.5 h) was recorded by *Z. piperitum* oil +10% vanillin. The mixtures of oils were not found to be so effective. Volatile compounds citronellal, citronellol, and geranio were isolated from essential oil of *Cymbopogon winterianus* (Java citronella) and made into a air freshener gel for slow release of volatile compound. Geraniol and citronellol recorded 78 and 77% repellent activity, respectively and the activity was maintained up to 16.82 and 12.77 days for geraniol and citronellol, respectively in gel form (Eden et al., 2020).

## CONCLUSIONS

Plant volatile oils and volatile compounds are promising mosquito control products. They are generally safe to human beings and other mammals. The mosquito control property of volatile oils is due to the presence of volatile compounds. From this review it is clear that some compounds like 1,8-cineole, linalool, limonene, eugenol and thymol have been frequently reported as effective larvicides and repellents against mosquitoes. Research on improving the efficacy of volatile oils is currently getting more importance. More research is needed for developing novel volatile oil formulations with controlled release technology and broad range activity against all vector mosquito species.

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