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Larvicidal efficacy of monoterpenes against the larvae of *Anopheles gambiae*

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ABSTRACT

Objective: To evaluate the larvicidal efficacy of eight volatile components of essential oils against 3rd instar larvae of *Anopheles gambiae s.s.*

Methods: Larvicidal effects of each compound were evaluated in both laboratory and semi-field trials. Stock solution was prepared and serial dilutions were made in six concentrations for each compound. A total of 20 larvae were exposed to larvicides for each replicate and monitored at intervals of 12, 24, 48 and 72 h. Larvae monitoring was done on basis of dead and live larvae in all intervals.

Results: All assayed compounds were larvicides and presented varying degrees of larval toxicity, with LC_{50} values ranging from 1.28 to 1 938.92 mg/L depending on the treatment time (12, 24, 48 or 72 h). (−)-Perillyl alcohol presented the strongest larvicidal activity towards *Anopheles gambiae* larvae, with LC_{50} values of 73.60, 18.36, 1.72 and 1.28 mg/L after 12, 24, 48 and 72 h of exposure, respectively. The next strongest were (−)-isopulegol (LC_{50} = 135.10, 49.39, 34.39 and 20.22 mg/L) and (−)-carvone epoxide (LC_{50} = 168.86, 124.74, 80.84 and 23.46 mg/L). After 12, 24 and 48 h of treatment, hydroxydihydrocarvone was the least toxic compound, with LC_{50} values of 1 938.92, 1 172.18 and 401.03 mg/L, respectively.

Conclusions: The data obtained in this study suggest that all evaluated monoterpenes, especially (−)-perillyl alcohol, have remarkable larvicidal effects and may be considered as potential sources for the development of suitable natural larvicides for mosquito management programs. Further small-scale field trials should be conducted.

1. Introduction

Mosquitoes constitute an important group of arthropods for public health. They transmit a wide range of human diseases such as filariasis, malaria, dengue, yellow fever and Japanese encephalitis, causing millions of deaths worldwide each year [1,2]. Global patterns of climate change and urbanization have increased the threat of humans contracting arthropod-borne viral infections [3].

Malaria is among the most important vector-borne diseases, being endemic to more than 100 countries worldwide, particularly in tropical and subtropical regions [4]. The disease is caused by one-celled parasites that are transmitted to humans via the bite of infected anopheline mosquitoes such as *Anopheles gambiae s.s.* Giles (*An. gambiae s.s.*), *Anopheles arabiensis* Patton and *Anopheles stephensi* Liston [5]. In the last 30 years, malaria incidence has increased, due mainly to the emergence of drug and insecticide resistance in parasites and vectors, respectively, as well as poor socioeconomic conditions [6]. But

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in the recent past, malaria vector and parasite populations have declined drastically due to increased investments in intervention, diagnosis and treatment [7,8].

Plants are a rich resource of alternative synthetic compounds for the control of mosquito larvae. They possess a wide range of bioactive phytochemicals that are selective, biodegradable, and have minor or no adverse effects on non-target organisms and the environment, making them potentially appropriate for use in integrated pest management programs. Approximately 2000 species of terrestrial plants have been described for their insecticidal properties [9–11].

Various studies have focused on the use of natural products, especially plant-derived essential oils, as suitable bioactive agents against the larvae of *An. gambiae s.s.* and other mosquito species [12–15]. Essential oils are complex natural mixtures of volatile organic compounds, principally mono- and sesquiterpenes, which are considered to be among the best alternatives for the control of disease vectors [16,17].

The present study investigated the larvicidal effects of eight monoterpenes found in volatile oils against the malaria vector mosquito *An. gambiae s.s.*

2. Materials and methods

2.1. Mosquito larvae

The *An. gambiae s.s.* larvae used in laboratory and semi-field assays were obtained from the insectary of the Tropical Pesticides Research Institute. Only 3rd instar larvae were used, according to World Health Organization protocol [18]. Larval rearing in the insectary was carried out according to the protocol developed by Balestrino *et al.* [19]. Larvae were reared at (27.0 ± 2.0) °C, a photoperiod of 12:12 h (light: dark), and (78 ± 2)% relative humidity. Larvae were fed a diet of TetraMin fish food.

2.2. Larval assays in the laboratory

The assayed compounds (−)-perillyl alcohol, (−)-isopulegol, (+)-limonene epoxide, (+)-limonene, terpinen-4-ol, and terpinolene were acquired from Sigma–Aldrich, USA. The (−)-carvone epoxide [20] and (−)-hydroxydihydrocarvone [21] were prepared as previously described.

Larvicidal bioassays were conducted as described by Mdoe *et al.* [12,13]. A stock solution was prepared for each test compound by dissolving the compound in 98 mL normal laboratory larval rearing water and 2 mL dimethylsulfoxide (DMSO) in a 100 mL plastic container. The solution was thoroughly mixed to get a homogeneous mixture, and serial dilutions of 200, 100, 50, 25 and 12.5 mg/L were prepared. Each experiment was replicated at least six times with two controls: one containing normal laboratory larval rearing water, the other containing an aqueous solution of 1% DMSO to evaluate the effect of the solvent on the larvae. For the larvicidal experiments, each replicate and each control received 20 live 3rd instar larvae. No nutritional supplements were added during the assays. Larval mortality was registered after 12, 24, 48 and 72 h of exposure. The larvae were considered dead if they did not present movement.

2.3. Larval assays in the semi-field

Semi-field larvae bioassays were conducted using the same concentrations used in the laboratory assays. Semi-field environment structures used in this study were designed according to previous studies [12,22] and following World Health Organization recommendations [18]. Each experiment was carried out in six replicates with two controls, one having an aqueous solution of 0.5% DMSO and the other having normal laboratory larval rearing water. For the larvicidal assay, 20 live 3rd instar larvae were placed in each assay replicate and in each control.

2.4. Statistical analysis

Scheffé's multiple comparison procedure was used to determine the statistical significance of the larvicidal activity of the tested compounds, with results expressed as mean ± SE. Statistical analysis was performed using SAS. Assessments of surviving larvae were recorded after 12, 24, 48 and 72 h of exposure. Mortality was reported as LC_{50}, the concentration that produced 50% mortality. The 95% confidence intervals (*CI*) for LC_{50} were also recorded.

3. Results

In this study, the larvicidal toxicity of a series of eight monoterpenes (Figure 1) present in volatile oils was evaluated against 3rd stage larval instars of *An. gambiae s.s.*, one of the most anthropophilic vectors of malaria. Larval mortality rates were registered after 12, 24, 48 and 72 h of treatment in varying concentrations of the test solutions. The result of each bioassay was reported as the lethal concentration estimated to kill 50% of the treated larvae (LC_{50}), expressed in mg/L. The LC_{50} values for each compound and treatment time, along with 95% *CI*, were given in Table 1.

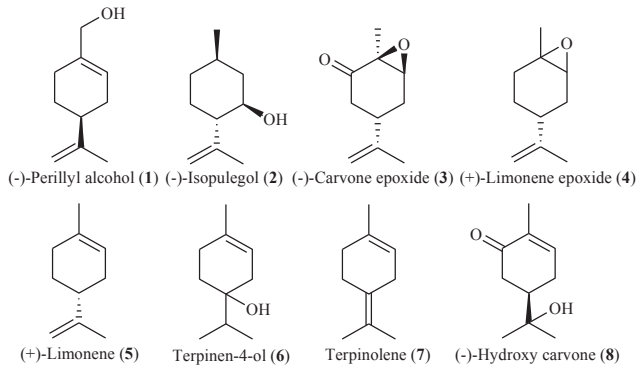

Figure 1. Chemical structures of the evaluated compounds.

All the assayed compounds had larvicidal effects and exhibited different degrees of larval toxicity, with LC_{50} values varying between 1.28 and 1938.92 mg/L depending on the treatment time (12, 24, 48 or 72 h). Among the eight monoterpenes, (−)-perillyl alcohol (1) showed the strongest larvicidal activity towards *An. gambiae* larvae, with LC_{50} values of 73.60, 18.36, 1.72 and 1.28 mg/L after 12, 24, 48 and 72 h of exposure, respectively. The next strongest were (−)-isopulegol (2) (LC_{50} = 135.10, 49.39, 34.39 and 20.22 mg/L) and (−)-carvone

Table 1
LC_{50} (mg/L) and 95% *CI* of the compounds 1–8.

Compound	LC_{50} (*CI*)			
	12 h	24 h	48 h	72 h
1	73.60 (55.72–95.19)	18.36 (12.47–25.46)	1.72 (0.70–3.64)	1.28 (0.44–3.12)
2	135.10 (105.31–169.97)	49.39 (37.08–63.90)	34.39 (25.92–44.32)	20.22 (14.99–26.41)
3	168.86 (132.49–210.61)	124.74 (92.10–162.61)	80.84 (61.60–102.92)	23.46 (17.34–30.52)
4	276.56 (231.69–329.53)	200.85 (168.20–239.31)	118.59 (98.55–141.79)	88.60 (74.36–105.03)
5	334.02 (274.48–402.33)	270.34 (214.18–332.34)	152.95 (124.35–184.84)	97.72 (74.12–123.73)
6	509.89 (406.23–629.01)	337.73 (269.27–417.21)	293.13 (227.25–365.72)	194.29 (144.99–246.40)
7	493.38 (374.42–628.60)	404.71 (298.75–517.64)	343.79 (247.73–441.02)	259.40 (172.94–340.36)
8	1 938.92 (1 307.20–3 167.95)	1 172.18 (818.87–1 777.14)	401.03 (231.59–595.63)	50.95 (33.76–70.53)

epoxide (3) (LC_{50} = 168.86, 124.74, 80.84 and 23.46 mg/L). After 12, 24 and 48 h of treatment, (−)-hydroxydihydrocarvone (8) was the least toxic compound, with LC_{50} values of 1 938.92, 1 172.18 and 401.03 mg/L, respectively. Terpinolene (7) exhibited the lowest toxicity at 72 h post-treatment, with an LC_{50} value of 259.40 mg/L.

Larval mortality rates were found to be directly proportional to monoterpene concentrations. Similarly, larval mortality increased with increasing exposure time, as all assayed compounds showed the highest mortality rates after 72 h of treatment. The most remarkable result was seen with (−)-hydroxydihydrocarvone (8), which was 38-fold more bioactive at 72 h post-treatment (LC_{50} = 50.95 mg/L) than at 12 h (LC_{50} = 1 938.92 mg/L). To better understand the relationship between the molecular structure of the assayed monoterpenes and their larval toxicity, specific structural and functional group variations were identified as possibly contributing to larvicidal activity. In general, the oxygenated monoterpenes exhibited stronger larvicidal effects than the monoterpene hydrocarbon (+)-limonene (5). The position of the carbon–carbon double bond in the *p*-menthane skeleton appeared to influence larvicidal potency; after 48 and 72 h of treatment time, (+)-limonene (5) (LC_{50} = 152.95 and 97.72, respectively) was more bioactive than terpinolene (7) (LC_{50} = 343.79 and 259.40, respectively). Similarly, the position of the hydroxyl group (endo- or exocyclic) also altered the toxicity, as seen in the monoterpenoids (−)-perillyl alcohol (1), (−)-isopulegol (2) and terpinen-4-ol (6), which each exhibited different degrees of larval toxicity. Furthermore, replacement of a C–C double bond by an epoxide group did not significantly affect larvicidal potency, as (+)-limonene epoxide (4) and (+)-limonene (5) showed similar activity. However, the addition of a ketone group in the cyclohexane ring seemed to contribute to larvicidal efficacy, as seen in the relatively high bioactivity of (−)-carvone epoxide (3) compared to (+)-limonene epoxide (4) and (+)-limonene (5).

4. Discussion

The findings of this study have shown that, compounds with different orientations of the active groups in primary structure influence the outcome of larvae mortality differently. These results are interesting, since the evaluated compounds are highly volatile. Several studies in the literature have described the larvicidal activity of monoterpenes against different species of mosquitoes [17,23–27]. Perumalsamy *et al.* reported the larvicidal potential of the monoterpenes camphene, fenchone, terpinolene, γ-terpinene, (+)- and (−)-β-pinene, (+)- and (−)-α-pinene, α-terpineol, myrcene, terpinen-4-ol, (+)-limonene, Δ^3-carene, borneol, 1,8-cineole, linalool, verbenone and α-phellandrene against three mosquito species, *Culex pipiens pallens*, *Aedes aegypti* and *Ochlerotatus togoi* [28]. In another study, Tabanca *et al.* found that (−)-perillyl alcohol, (−)-perilla aldehyde, (−)-perillic acid and (−)-limonene exhibited high toxicity against 3rd instar larvae of *Aedes aegypti*, with LC_{50} values of 39.1, 35.3, 56.5 and 29.1 mg/L, respectively [29]. Liu *et al.* showed that the monoterpenes (+)-limonene and geraniol, both isolated from the essential oil from the roots of *Toddalia asiatica* (L.) Lam., displayed an interesting larvicidal activity against 3rd instar larvae of *Aedes albopictus*, with LC_{50} values of 19.84 and 30.13 μg/mL, respectively [16].

The findings of the current study suggest possibilities for further research on the larvicidal activity of plant-derived essential oils and their chemical components. Future studies should focus on developing more stable and effective formulations, investigating the mode of the constituents' actions, decreasing costs, and examining the effects of these compounds on non-target organisms and the environment [6,30,31]. The compounds are found in essential oils of plants, such as *Conyza newii* (perillyl alcohol and limonene) [32], *Eucalyptus citriodora* (isopulegol) [33], *Carum carvi* (carvone epoxide) [34], *Artemisia nilagirica* var. *septentrionalis* (terpinen-4-ol) [35], lemon (limonene-1,2-epoxide) [36], *Mangifera indica* L. (terpinolene) [37], and *Nicotiana tabacum* (hydroxydihydrocarvone) [38]. These monoterpenes toxicity against mosquito larvae have shown the prospect of replacing synthetic larvicides which are losing efficacy or been incorporated in integrated vector control management programme.

All of the tested monoterpenes exhibited larvicidal activity against *An. gambiae s.s.*, with (−)-perillyl alcohol the most toxic after 12, 24, 48 and 72 h of treatment. These results underscore the importance of evaluating plant essential oils and their chemical components as effective natural larvicides for controlling *Anopheles* larvae, especially in areas where vectors have developed resistance or diminished susceptibility to conventional synthetic insecticides.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Das NG, Goswami D, Rabha B. Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *J Vector Borne Dis* 2007; **44**: 145-8.

[2] Pugazhvendan SR, Elumali K. Larvicidal activity of selected plant essential oil against important vector mosquitoes: dengue vector, *Aedes aegypti* (L.), malarial vector, *Anopheles stephensi* (Liston) and filarial vector, *Culex quinquefasciatus* (Say) (Diptera: Culicidae). *Middle East J Sci Res* 2013; **18**(1): 91-5.

[3] Intirach J, Junkum A, Tuetun B, Choochote W, Chaithong U, Jitpakdi A, et al. Chemical constituents and combined larvicidal effects of selected essential oils against *Anopheles cracens* (Diptera: Culicidae). *Psyche* 2012; **2012**: 591616.

[4] Rajkumar S, Jebanesan A, Nagarajan R. Effect of leaf essential oil of *Coccinia indica* on egg hatchability and different larval instars of malarial mosquito *Anopheles stephensi*. *Asian Pac J Trop Med* 2011; **4**: 948-51.

[5] Wachira SW, Omar S, Jacob JW, Wahome M, Alborn HT, Spring DR, et al. Toxicity of six plant extracts and two pyridone alkaloids from *Ricinus communis* against the malaria vector *Anopheles gambiae*. *Parasit Vectors* 2014; **7**: 312.

[6] Vatandoost H, Dehakia M, Djavadia E, Abai MR, Duchson S. Comparative study on the efficacy of lambdacyhalothrin and bifenthrin on torn nets against the malaria vector, *Anopheles stephensi* as assessed by tunnel test method. *J Vector Borne Dis* 2006; **43**: 133-5.

[7] Meyrowitsch DW, Pedersen EM, Alifrangis M, Scheike TH, Malecela MN, Magesa SM, et al. Is the current decline in malaria burden in sub-Saharan Africa due to a decrease in vector population? *Malar J* 2011; **10**: 188.

[8] Mmbando BP, Vestergaard LS, Kitua AY, Lemnge MM, Theander TG, Lusingu JP. A progressive declining in the burden of malaria in North-eastern Tanzania. *Malar J* 2010; **9**: 216.

[9] Nanyonga SK, Opoku A, Lewu FB, Oyedeji AO. Chemical composition and larvicidal activity of the essential oil of *Tarchonanthus camphoratus* against *Anopheles arabiensis* mosquito larvae. *J Essent Oil Bear Plants* 2012; **15**: 288-95.

[10] Cheng SS, Lin CY, Chung MJ, Liu YH, Huang CG, Chang ST. Larvicidal activities of wood and leaf essential oils and ethanolic extracts from *Cunninghamia konishii* Hayata against the dengue mosquitoes. *Ind Crops Prod* 2013; **47**: 310-5.

[11] Shivakumar MS, Srinivasan R, Natarajan D. Larvicidal potential of some Indian medicinal plant extracts against *Aedes aegypti* (L.). *Asian J Pharm Clin Res* 2013; **6**: 77-80.

[12] Mdoe FP, Cheng SS, Lyaruu L, Nkwengulila G, Chang ST, Kweka EJ. Larvicidal efficacy of *Cryptomeria japonica* leaf essential oils against *Anopheles gambiae*. *Parasit Vectors* 2014; **7**: 426.

[13] Mdoe FP, Cheng SS, Msangi S, Nkwengulila G, Chang ST, Kweka EJ. Activity of *Cinnamomum osmophloeum* leaf essential oil against *Anopheles gambiae s.s*. *Parasit Vectors* 2014; **7**: 209.

[14] Pavela R, Kaffkova K, Kumsta M. Chemical composition and larvicidal activity of essential oils from different *Mentha* L. and *Pulegium* species against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Plant Prot Sci* 2014; **50**: 36-42.

[15] Ali A, Tabanca N, Demirci B, Blythe EK, Ali Z, Baser KH, et al. Chemical composition and biological activity of four salvia essential oils and individual compounds against two species of mosquitoes. *J Agric Food Chem* 2015; **63**: 447-56.

[16] Liu XC, Dong HW, Zhou L, Du SS, Liu ZL. Essential oil composition and larvicidal activity of *Toddalia asiatica* roots against the mosquito *Aedes albopictus* (Diptera: Culicidae). *Parasitol Res* 2013; **112**: 1197-203.

[17] Dias CN, Moraes DF. Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides: review. *Parasitol Res* 2014; **113**: 565-92.

[18] World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization; 2005. [Online] Available from: http://apps.who.int/iris/bitstream/10665/69101/1/WHO_CDS_WHOPES_GCDPP_2005.13.pdf [Accessed on 20th September, 2015]

[19] Balestrino F, Benedict MQ, Gilles JR. A new larval tray and rack system for improved mosquito mass rearing. *J Med Entomol* 2012; **49**: 595-605.

[20] Salgado PR, da Fonsêca DV, Braga RM, de Melo CG, Andrade LN, de Almeida RN, et al. Comparative anticonvulsant study of epoxycarvone stereoisomers. *Molecules* 2015; **20**: 19660-73.

[21] Buechi G, Wueest H. New synthesis of beta agarofuran and of dihydroagarofuran. *J Org Chem* 1979; **44**: 546-9.

[22] Kweka EJ, Nyindo M, Mosha F, Silva AG. Insecticidal activity of the essential oil from fruits and seeds of *Schinus terebinthifolia* Raddi against African malaria vectors. *Parasit Vectors* 2011; **4**: 129.

[23] Evergetis E, Michaelakis A, Kioulos E, Koliopoulos G, Haroutounian SA. Chemical composition and larvicidal activity of essential oils from six Apiaceae family taxa against the West Nile virus vector *Culex pipiens*. *Parasitol Res* 2009; **105**: 117-24.

[24] Zhu L, Tian YJ. Chemical composition and larvicidal effects of essential oil of *Blumea martiniana* against *Anopheles anthropophagus*. *Asian Pac J Trop Med* 2011; **4**: 371-4.

[25] Tripathi AK, Prajapati V, Ahmad A, Aggarwal KK, Khanuja SPS. Piperitenone oxide as toxic, repellent, and reproduction retardant toward malarial vector *Anopheles stephensi* (Diptera: Anophelinae). *J Med Entomol* 2004; **41**: 691-8.

[26] Lucia A, Zerba E, Masuh H. Knockdown and larvicidal activity of six monoterpenes against *Aedes aegypti* (Diptera: Culicidae) and their structure-activity relationships. *Parasitol Res* 2013; **112**: 4267-72.

[27] Michaelakis A, Vidali VP, Papachristos DP, Pitsinos EN, Koliopoulos G, Couladouros EA, et al. Bioefficacy of acyclic monoterpenes and their saturated derivatives against the West Nile vector *Culex pipiens*. *Chemosphere* 2014; **96**: 74-80.

[28] Perumalsamy H, Kim NJ, Ahn YJ. Larvicidal activity of compounds isolated from *Asarum heterotropoides* against *Culex pipiens* pallens, *Aedes aegypti*, and *Ochlerotatus togoi* (Diptera: Culicidae). *J Med Entomol* 2009; **46**: 1420-3.

[29] Tabanca N, Demirci B, Ali A, Ali Z, Blythe EK, Khan IA. Essential oils of green and red *Perilla frutescens* as potential sources of compounds for mosquito management. *Ind Crops Prod* 2015; **65**: 36-44.

[30] Cheng SS, Huang CG, Chen YJ, Yu JJ, Chen WJ, Chang ST. Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species. *Bioresour Technol* 2009; **100**: 452-6.

[31] Liu XC, Liu Q, Zhou L, Liu ZL. Evaluation of larvicidal activity of the essential oil of *Allium macrostemon* Bunge and its selected major constituent compounds against *Aedes albopictus* (Diptera: Culicidae). *Parasit Vectors* 2014; **7**: 184.

[32] Mayeku WP, Omollo NI, Odalo OJ, Hassanali A. Chemical composition and mosquito repellency of essential oil of *Conyza newii* propagated in different geographical locations of Kenya. *Med Vet Entomol* 2014; **28**(3): 253-6.

[33] Olivero-Verbel J, Nerio LS, Stashenko EE. Bioactivity against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) of *Cymbopogon citratus* and *Eucalyptus citriodora* essential oils grown in Colombia. *Pest Manag Sci* 2010; **66**(6): 664-8.

[34] Iacobellis NS, Lo Cantore P, Capasso F, Senatore F. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. *J Agric Food Chem* 2005; **53**: 57-61.

[35] Haider F, Kumar N, Naqvi AA, Bagchi GD. Oil constituents of *Artemisia nilagirica* var. *septentrionalis* growing at different altitudes. *Nat Prod Commun* 2010; **5**(12): 1959-60.

[36] Blanch GP, Nicholson GJ. Determination of the enantiomeric composition of limonene and limonene-1,2-epoxide in lemon peel by multidimensional gas chromatography with flame-ionization detection and selected ion monitoring mass spectrometry. *J Chromatogr Sci* 1998; **36**: 37-43.

[37] Ramos EH, Moraes MM, Nerys LL, Nascimento SC, Militão GC, de Figueiredo RC, et al. Chemical composition, leishmanicidal and cytotoxic activities of the essential oils from *Mangifera indica* L. var. Rosa and Espada. *Biomed Res Int* 2014; **2014**: 734946.

[38] Demole E, Berthet D. Chemical study of Burley tobacco flavor (*Nicotiana tabacum* L.). I. Volatile to medium-volatile constituents. *Helv Chim Acta* 1972; **55**(6): 1866-82.