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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.

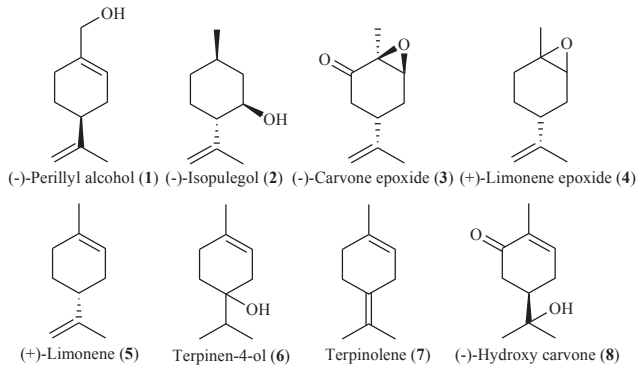

Figure 1. Chemical structures of the evaluated compounds.

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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