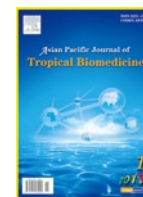




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Repellent activity of *Eucalyptus* and *Azadirachta indica* seed oil against the filarial mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) in India

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ABSTRACT

Objective: To evaluate the repellent activity of *Eucalyptus* and *Azadirachta indica* (*A. indica*) seed oil against filarial mosquito *Culex quinquefasciatus* (*Cx. quinquefasciatus*) from Purulia district of the West Bengal state, India. **Methods:** The repellent activity of *Eucalyptus* and *A. indica* seed oils (using coconut oil base) against *Cx. quinquefasciatus* mosquito were evaluated in indoor conditions. Three concentrations, 0%, 50% and 100% (v/v) of both the agents were considered in the studies. The protection percentage was determined, and the protection time was recorded. **Results:** The test oils showed excellent repellent action against *Cx. quinquefasciatus*. The *A. indica* seed oil provided 90.26% and 88.83% protection, and the *Eucalyptus* oil 93.37% and 92.04%, at concentrations 50% and 100% (v/v), respectively, with the protection time up to 240 min. There was no bite within 120 min and 180 min, respectively, due to the action of *Eucalyptus* and *A. indica* seed oil, and thus 100% protection from the bite of *Cx. quinquefasciatus* mosquito was achieved. **Conclusions:** The present study clearly demonstrates the potential of *Eucalyptus* and *A. indica* seed oils as topical repellents against *Cx. quinquefasciatus*, the mosquito vector of filariasis.

1. Introduction

The mosquitoes are a serious threat to public health, since they are known vector of many life-threatening diseases like malaria, filariasis, Japanese encephalitis, chikungunya and dengue fever, which are transmitted by the species of three mosquito genera: *Anopheles*, *Culex* and *Aedes*. Repellency is an important way of preventing vector-borne diseases by reducing man-vector contact. Most commercial repellents are prepared by using chemicals like N, N-diethyl-3-methylbenzamide (DEET), dimethyl phthalate (DMP) and allethrin, and the most common mosquito repellents available in the market contain DEET [1]. The effective dose of DEET has been documented as 0.000 7 mg/cm² for *Aedes aegypti* (*Ae. aegypti*), *Anopheles maculatus* (*An. maculatus*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) [2].

But, these chemical repellents are not safe for public use because of their potential toxicity. Moreover, extensive use of the chemical agents for the control of vector-

borne diseases has created problems related to adverse environmental effects, high operational cost, community acceptance and physiological resistance to vectors [3]. The mosquito *Cx. quinquefasciatus* Say (Family: *Culicidae*) is the potential vector of bancroftian filariasis throughout the world including India, but the control of *Cx. quinquefasciatus*-borne diseases are becoming increasingly difficult due to the reasons mentioned above. It is needful to develop environmentally friendly, long-lasting, low cost and preferably locally available repelling agents for mosquito control.

The biologically active materials derived from plant sources have been reported either as insecticides for killing larvae or adult mosquitoes, or as repellents for mosquito biting. They are one of the best alternatives for mosquito control [4, 5]. Most plant-based insect repellents contain plant oils including *Eucalyptus* and *Azadirachta indica* (*A. indica*) oils. Preliminary studies suggest that *Eucalyptus* oil provides longer protection than other available plant-based repellents [6].

The oils extracted from turmeric (*Curcuma longa*), citronella grass (*Cymbopogon winterianus*) and hairy basil (*Ocimum americanum*) repelled the mosquito vectors *Ae.*

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aegypti, *An. dirus* and *Cx. quinquefasciatus* for up to 8 h, while the oil derived from kaffir lime (*Citrus hystrix*) was effective for 3 h. Essential oil from *Piper aduncum* gave stronger complete protection than *Zanthoxylum piperitum* against *Ae. aegypti* for 1.5 hours after application [7]. The application of *Mentha piperita* essential oil provided 150 min, 100% protection against *Ae. aegypti* [8]. However, no scientific study reported repellent activity of plant products against mosquito vectors from this part of our country. Herein, we conducted an indoor study in order to determine the repellent activity of *Eucalyptus* and *A. indica* oils against *Cx. quinquefasciatus* at Purulia district of the West Bengal state, India.

2. Materials and method

2.1. Repellents

The *Eucalyptus oil* (Indian Drug House, Sonarpur, India) procured from the market, and the *A. indica* seed oil collected from villagers at Kallabera of Purulia district, West Bengal state, India were used as the repellents in the present study. Dilutions of the stock oils were made with coconut oil (v/v) at 50% concentration. The negative and positive control agents were 100% coconut oil plus 0% *Eucalyptus* oil, and 100% *Eucalyptus* oil plus 0% coconut oil, respectively. Similar concentrations were prepared with *A. indica* seed oil and subjected to the study.

2.2. Repellency test

Aliquot of 5 mL of the test solution was smeared on exposed parts of hands and legs of the human volunteers, after the hands and legs were washed and cleaned thoroughly with rectified spirit. The subject was then allowed to sit for 30 min, on a chair in a room of the house at Kallabera, west Bengal (India), with stretched hands and legs in order to facilitate the female mosquitoes to bite. The tests were performed repeatedly for 4 h at 30 min intervals (between 8: 00 pm and 12: 00 pm) using *Eucalyptus* oil at 0%, 50% and 100% concentrations, as prepared above. Any mosquito perching on the exposed parts was caught before it fed (by inverting a small glass tube over it, in order to identify the mosquitoes). Similar studies were performed with *A. India* seed oil at all the concentrations under the criteria mentioned above. Mosquitoes collected before the application of the repelling agents was taken as the control.

The protection percentage was calculated according to the standard procedure described by Ansari *et al* [9]: protection percentage = $[(C-T) / C] \times 100$, where C is the number of mosquitoes collected from the controls and T is the number of collected mosquitoes from the treated baits.

2.3. Statistical analysis

The results from the treated and untreated baits (both at 50% and 100%) were statistically analyzed using χ^2 tests. The protection percentages of the test agents at 50% and 100% concentrations for 4 h were also compared and the χ^2 tests were followed to compare the protection time of the two agents providing 100% protection.

3. Results

The repellent activity of *Eucalyptus* oil and *A. indica* seed oil against *Cx. quinquefasciatus* mosquito bites has been presented in Figure 1. The number of mosquitoes collected from experimental bait, treated with 50% and 100% of *A. indica* seed oil were 19 and 22, respectively, and the mosquitoes numbers from the control (untreated) bait were 195 and 197; For the *Eucalyptus* oil, 50 % and 100 %, the number of collected mosquitoes, respectively were 13 and 16 (from treated bait), and respectively 196 and 201 (from control bait).

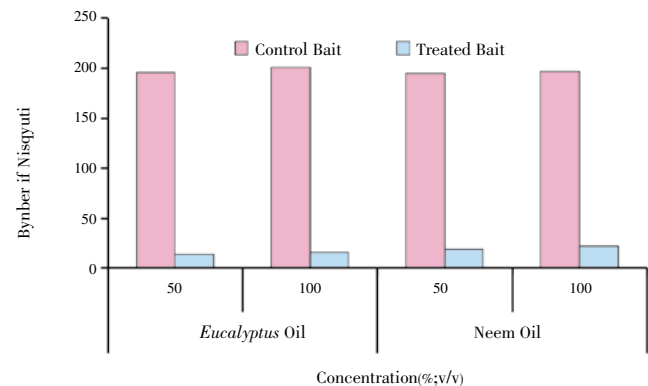


Figure 1. The *Cx. quinquefasciatus* mosquitoes collected from the experimental baits treated with different concentrations of *Eucalyptus* and *A. indica* seed oil.

The *A. indica* seed oil provided 90.26% and 88.83% protection, and the *Eucalyptus* oil provided 93.37% and 92.04%, when used at concentrations 50% and 100% for 4 h against *C. quinquefasciatus*. However, there was no bite within 180 min and 120 min, respectively, after applying *A. indica* seed oil and *Eucalyptus* oil, and thus 100% protection was achieved during these periods.

There was significant differences ($P < 0.001$) between the numbers of mosquitoes collected from the untreated and treated baits (with neem oil as well as *Eucalyptus* oil). Significant difference was found in protection time ($P < 0.001$) of the two agents providing 100% protection, but no significant difference was present in the protection percentage between the two concentrations (50% and 100%) of the two agents.

4. Discussion

In recent times, the use of environmentally friendly, biodegradable insecticides from plants to control insect vectors of diseases is gaining importance [10], because these botanicals have been found to be effective, user-friendly and inexpensive [11]. Kweka *et al* [12] reported that the repellency of oils extracted from plants had a high protection (83%–91%) against mosquito biting. Recently, essential oils have been studied for potential bioactive compounds. Applying *Piper aduncum* essential oil on human skin (25 cm² area), at a concentration of 0.4 g (400 μ L) resulted in 100% reduction in *Ae. aegypti* mosquito bite up to 2 hrs, and this protection was reduced to 70.03% at 8 hours' post-

treatment [13]. In search of alternative and safe methods of protection from mosquito bites, *A. indica* products were found to be effective mosquito repellents. These agents had 90%–100% protection against malaria vectors, and about 70% against *Cx. quinquefasciatus* [14]. Thavara *et al* [15] reported that the phytochemicals provided protection for 7 h against *Ae. aegypti*, and at least 8 h against *Cx. quinquefasciatus* and *An. dirus* under laboratory conditions. The essential oils from plants can be regarded as standard repellents, and the *Eucalyptus* oil must be re-applied every 123 min against *Culex mosquitoes* [16]. The current study determined the comparative repellent effects of *Eucalyptus* and *A. indica* seed oil against the filarial mosquito *Cx. quinquefasciatus* using human volunteers.

The repellency of *Eucalyptus* oil has been tested against three mosquito species: *Ae. albopictus*, *Ae. aegypti*, and *Cx. pipiens* [17]. Essential oil extracted from dried fruits of *Eucalyptus camaldulensis* (*E. camaldulensis*) has been tested for their repellency against *Cx. pipiens* [18]. The *Eucalyptus* oil (15%) gave protection to humans for 3 h against *Ae. albopictus* bite, and the protection time was prolonged to 5 h after adding 5% vanillin [19]. Skin repellency test at 1, 2.5 and 5 mg/cm² concentrations of *Cymbopogon citratus* gave 100% protection up to 3, 4 and 5 h, respectively, while the total protection percentage of the essential oil was recorded as 49.64% at 1 mg/cm², 62.19% at 2.5 mg/cm² and 74.03% at 5 mg/cm², against *Cx. quinquefasciatus* for 12 h [20]. Mosquitocidal and repellent actions of neem oil have been evaluated earlier [14, 21, 22]. Das *et al* [23] reported protection against the bites of mosquitoes for 7.4, 6.5 and 6.4 h with 60% (0.57 mg/cm²) concentration of essential oil of *Zanthoxylum armatum* (fruits), *Curcuma aromatica* (rhizomes) and oil of *A. indica*, respectively, in mustard oil base in field conditions. In the current study, the test agents (*Eucalyptus* oil and *A. indica* seed oil) both at 50% (with coconut oil base) and 100% (without base) concentrations, exhibited excellent repellency against *Cx. quinquefasciatus* bites.

The repellency percentages of the oils from *Eucalyptus* and *A. indica* at concentrations 50% (v/v) and 100% (v/v) are different, which suggests that the latter presented some hindrances to the expression of its repellent activity. The current findings are in conformity with those of earlier authors who documented repellent activity of different plant oils against mosquitoes [24–26]. This is due to the variety of essential oils, and the methods used. In addition, different plants have different compounds and volatility properties [27]. Bream *et al* [28] reported that the repellent action of the plant extracts tested varied depending on the plant parts, solvent used in extraction and the dose of the extract. The most effective plant extracts that had 100% repellency were petroleum ether extracts of the leaf, stem and root of *Echinochloa stagninum* at 5, 5 and 4.3 mg/cm².

Most plant essential oils and extracts are effective not for a long period of time because they are volatile and act on mosquitoes in the vapor phase [13, 29]. Many researches demonstrated improved repellency of plant-derived topical repellents after formulation with some bases or fixative materials, viz., liquid paraffin, vanillin, salicylic acid, and used the neem products as mosquito control agents [24–26, 30]. In the current study, though no remarkable difference in protection percentage was recorded between the pure (100% concentration) and diluted (50% in coconut oil) forms of the *Eucalyptus* oil (protection difference 1.33%) as well as *A.*

indica seed oil (protection difference 1.43%), it is interesting to note that, the test repellents, *Eucalyptus* and *A. indica* seed oil provided 100% protection from mosquito bites for 120 min and 180 min, respectively ($P < 0.001$). Tawastin *et al* [25] reported the repellent activity of turmeric, kaffir lime, citronella grass and hairy basil with the addition of 5% vanillin which prolonged the protection time up to 6 h. The plant oils at 30% concentration in mustard oil base provided protection for 296–304 min against *Ae. albopictus*, while the agents in coconut oil are protective for 223.5–245 min at the same concentration [31]. Application of 2% and 5% neem oil at 5 mL/person/night gave 50% and 40.9% protection in indoor collections and 17.4% and 5.6% in outdoor collections as compared with that of the untreated control, respectively. The protection time ranged from one to three hours and one to six hours in indoor and outdoor collections, respectively [32].

Nagpal *et al* [14] reported that *An. culicifacies* and *Cx. quinquefasciatus* mosquitoes were unable to bite the protected person within 4 h after his or her application of neem products, which were safe, and better than any other repellents without adverse reactions. The repellency percent at different observation periods (0, 1, 2, 4 and 6 h) ranged from 80% to 100% at different concentrations of *Cyperus rotundus* extract against *An. culicifacies*, *An. stephensi* and *Cx. quinquefasciatus*, suggesting that the extract might be applied as an effective personal protective measure against mosquito bites [33–39]. The plant-based repellent was less effective than the synthetic repellents, viz., DEET [40]. However, extracts of locally available plant parts offer promising repellents against mosquito biting [41]. The main implications of the current study include that the local plants can be used as alternatives to the chemical insecticides in reducing filarial vector density, human-mosquito contact and the vectorial capacity of the mosquito *Cx. quinquefasciatus*. However, more field studies on human volunteers are required in order to evaluate the activity of *Eucalyptus* and *A. indica* seed oil. These studies will provide valuable information about and insights into the role of particular repellents in preventing mosquito biting.

Conflict of interest statement

We declare that we have no conflict of interest.

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