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Essential oils of a few aromatic plants and their potential as knockdown, repellent and adulticidal agent to the filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Abstract

Use of synthetic products for the control of mosquito vectors raises serious concern about their negative impact on the environment, non-target organisms and human safety. Alternatively, the use of several natural products, particularly plant based essential oils have gained attention due to their good efficacy and ecofriendly nature. The adult knockdown effect and repellent activities of essential oils derived from three selected plants on *Culex quinquefasciatus* were evaluated. Data processing was performed using probit analysis. The results showed that essential oils from all the selected plants exhibited considerable knockdown effect on *Cx. quinquefasciatus*. In addition to this, all plant derived essential oils showed repellent activity which was significant in all concentrations. It is suggested that essential oils derived from *Citrus limon* were found to be very active as mosquito repellent.

Keywords: Mosquito vector, essential oils, repellent activity, *Culex quinquefasciatus*, knockdown, *Citrus limon*, *Cinnamomum verum*, *Lantana camara*

Introduction

Mosquitoes are considered as the most concerned insect groups of public health importance owing to their capability of spreading several diseases [1, 2] and these mosquito-borne diseases induce major health problems in developing countries at large extent [3]. In addition to the spreading of diseases they are considered as human pests causing allergic responses including skin reactions [4]. In the past decades insecticides of chemical origin have been considered as effective against mosquito vectors and constant application of chemical insecticides including organophosphates is generally undertaken to eradicate mosquitoes [5]. The extensive and continuous application of these chemicals induced resistance in mosquitoes followed by the resurgence of mosquito-borne diseases [6]. Moreover, repeated usage of chemical insecticides leads to undesirable effects on non-target organisms [7, 8].

In the light of increased resistance of mosquitoes against chemical insecticides, plant derived compounds are used as an alternative to chemical insecticides [9]. Essential oils extracted from plants have been widely used as mosquito repellents and provide considerable protection from the deadly vector mosquitoes [10, 11, 12, 13]. Many studies have been supported the anti-insect potential of several plant based essential oils [14, 15, 16]. Essential oils have received much attention due to the presence of potential bioactive compounds which can be effectively used against insect pests [17].

Cheng *et al.* [18] reported that several essential oils exhibit strong mosquito repellent and larvicidal activity in addition to their promising inhibitory effects against bacteria, termites and fungi. Many of the active components isolated from plant extracts exert toxic activity against mosquito larvae [19, 20, 21, 22]. In addition to this they can also be used as ovicidal, oviposition deterrents, growth and reproduction inhibitors [23, 24] or adult repellents [25]. Here we report the knockdown and repellent activity of essential oils extracted from selected plants against *Culex quinquefasciatus* Say.

Materials and methods

Collection of plants

All the three plants tested are collected from in and around of Calicut University Campus, Malappuram, Kerala and are taxonomically identified from the Department of Botany, University of Calicut and presented in the table 1 below.

Test organism

Culex quinquefasciatus was selected as test organism. This species comes under *Culex pipiens* species complex and act as a potential vector of *Wuchereria bancrofti* causing filariasis and female mosquitoes host the filarial parasites.

Extraction of essential oil

Three aromatic plants were selected for essential oil extraction. Essential oils of selected plants were extracted by steam distillation method using Clevenger apparatus. The leaves of the plants were collected, washed and used for essential oil extraction using distilled water for 4 h. The resulting essential oils were dried over anhydrous-sodium sulfate and used to conduct bioassays.

Adulticidal bioassay

The adulticidal activity of different concentrations of the selected essential oils are also estimated. The bioassay was performed by following WHO [26] protocol. Different concentrations of the oils were prepared by dissolving the oil in ethanol and applied on Whatman No. 1 filter papers. Control papers were treated with ethanol and distilled water under similar conditions. Twenty female mosquitoes (2–5 days old glucose fed, blood starved) each were collected from the insect-rearing cage and gently transferred into a plastic holding tubes. The mosquitoes were allowed to acclimatize in

the tube for 1 h and then exposed to test paper (filter paper) for 1 h. At the end of exposure period, the mosquitoes were transferred back to the holding tube and kept for 24 h for recovery period. Mortality of mosquitoes was determined at the end of 24 hr. The number of mosquitoes knocked down in the exposure tube was recorded at 5 min interval period till the last mosquito was knocked down. Knock down time (KDT) values of KDT50 and KDT90 were determined using probit analysis.

Adult repellency test

The repellency activity was conducted with slightly modified method of WHO [27]. Repellency bioassays were carried out in the laboratory at 27-35°C and 60-80% RH. Three to four days old blood-starved 100 adult females of *Culex quinquefasciatus* mosquito was randomly selected and placed in an experimental cage (30 x 30 x 30 cm) and left to acclimatize for 1h. The arms of the tested persons were cleaned with ethanol. After air drying the arm of the test person, only 25 cm² dorsal side of the skin on each arm was exposed and the remaining area were covered with rubber gloves. The selected essential oils at different concentrations (50, 100, 150, 200 and 250 ppm) was applied. The control and treated arms were introduced simultaneously into the cage. The first bite by *Culex quinquefasciatus* was noted from 5 minutes for every 1h up to 6 h. Subsequently, the test arm was introduced into the cage for the same period of time and the number of mosquitoes that landed and attempted to feed were recorded. Each experiment was conducted three times. It was observed that there was no skin irritation by the extracts of the selected essential oils. The percentage protection was calculated by using the following formula;

$$\text{Percentage Protection} = \frac{\text{No. of bites received by control} - \text{No. of bites received by treated}}{\text{No. of bites received by control}} \times 100$$

Results

Among selected plant extracts *C. limon* exhibited higher knockdown effect at minimum concentration followed by *L. camara* and *C. verum*. It was noted that the knockdown effects of all plant extracts are positively correlated with the concentration (Fig.1). *C. limon* consistently exhibited higher knockdown effect in all tested concentrations. All plants exhibited similar trend of activity except *C. verum* was more active than *L. camara* at 1% concentration.

It was found that the mosquito repellent activity of selected plant extracts is distinct in all concentrations (Figs2-6). *Citrus limon* exhibited maximum repellent activity in all concentrations. The selected plants exhibited 100 percent repellent activity only for one hour in case of concentration below 50% (Figs 2 & 3). Later the repellent activity has been reduced gradually. From 50–70% concentration the repellent activity of all plants persisted up to three hours exposure time (Figs 4 & 5). At 10% *C. limon* showed maximum repellent activity followed by *C. verum* and *L. camara*. The difference in the activity of *C. verum* and *L. camara* is found to be distinct between 5–6 hours of exposure (Fig. 2). At 25% concentration the increase in activity of *C. verum* was evident between 2–5 hours whereas at 6hour exposure of *L. camara* and *C. verum* showed almost similar activities (Fig. 3).

The repellent activity of selected plant extracts was found to be consistent up to 3 hours of exposure at 50% concentration.

Similarly, to lower concentrations the activity of *C. verum* found higher than *L. camara* (Fig. 4). At 70% concentration *C. limon* and *C. verum* exhibited maximum repellent activity up to 5 hours of exposure. It is noted that the repellent activity of *L. camara* decreased drastically after 4 hours of exposure (Fig. 5). At 100% concentration all plant extracts showed maximum activity up to 5 hours of exposure (Fig. 6). Likewise, to all concentrations the activity of *L. camara* was decreased considerably than *C. verum* (Fig. 6).

Discussion

Plant derived essential oils have been widely used to ensure protection from mosquitoes and other blood sucking insects [28]. All essential oils from selected plants showed remarkable knockdown activity against *Cx. quinquefasciatus*. The results of the present study showed that *C. limon* exhibited higher knockdown effect in all concentrations than other essential oils tested. The increased activity of *C. limon* is supported by Soonwera [29] as essential oil from eight *Citrus* plants showed higher knockdown effect against *Cx. quinquefasciatus* and *Aedes aegypti*. *Citrus limon* exhibited higher knockdown activity in all the concentrations. The results showed that *Cx. quinquefasciatus* is found to be susceptible towards essential oils derived from plants particularly from *Citrus* plants [29]. This result is further supported by Tawatsin *et al.* [30] reporting essential oils from 18 plants belonging to 11 families found to

be active against *Cx. quinquefasciatus*. Apart from *C. limon*, other essential oils like *L. camara* and *C. verum* showed moderate activity against *Cx. quinquefasciatus*. The activities of these plants are in line with the results of Dua *et al.* [31] and Manimaran *et al.* [32] as essential oil from *L. camara* and *C. verum* found effective against three mosquito species (*An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*).

Several plant-derived essential oils have been found as mosquito repellent by reducing the biting nuisance [33-37]. In the present study it was observed that all the essential oils exhibited considerable repellent activity against *Cx. quinquefasciatus* at different concentrations. Among the selected plants, *C. limon* was found to be highly active. Essential oil from *C. limon* exhibited nearly 100% protection in all concentrations and the increased activity is comparable with that of *Citrus aurantifolia*, which exhibited an average of 98.3% protection from *Cx. quinquefasciatus*. In addition to this, the potential for using *Citrus* derived essential oils as an alternative to synthetic repellent cannot be neglected.

Essential oils from *Cinnamomum verum* showed repellent activity at 50% concentration and the activity was extended up to 6 hours. The differential activity of *Cinnamomum* is supported by Prajapati *et al.* [38] and reported that

Cinnamomum zeylanicum is found to be good candidate for using as repellent and adulticidal botanical against *Cx. quinquefasciatus*. It was noted that the 100% repellent activity of the essential oils derived from *Cinnamomum* spp. (*C. camphora* and *C. zeylanicum*) against *Anopheles stephensi* [39] and oils from *C. zeylanicum* exhibit one-hour protection from *An. Subpictus* [40]. Based on the effectiveness of essential oils derived from *Cinnamomum* species against *Cx. quinquefasciatus*, it may be used as an effective control method for other mosquitoes as well. Essential oil from *L. camara* showed repellent activity similar to that of *C. verum*. From the results of the present study, it was evident that essential oils derived from plants are found to be a good alternative to synthetic repellents with their promising knockdown and repellent activity against *Cx. quinquefasciatus*. However, for practical use of these essential oils, further research should be done to rule out if any problem still exist in order to address with human safety. Present study also delivers important information on the plant based essential oils and their differential activity against *Cx. quinquefasciatus*. Considering all these, it can be inferred that the essential oils derived from these plants could be used effectively against mosquito vectors.

Table 1: Lists of plants collected from different localities from the campus of the University of Calicut, Kerala

Name of the Plant	Common Name	Family	Part used
<i>Cinnamomum verum</i> Schaeff.	Cinnamon	Lauraceae	Leaves
<i>Citrus limon</i> (L.) Osbeck	Citrus	Rutaceae	Leaves
<i>Lantana camara</i> L.	Nil	Verbenaceae	Leaves

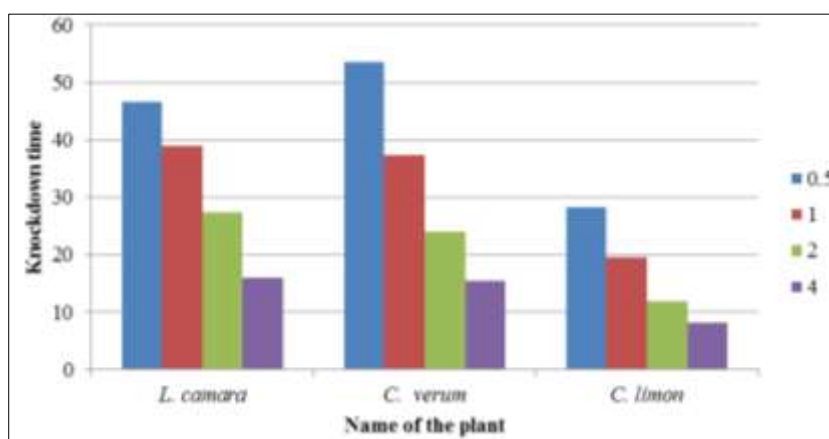


Fig 1: Knock down times required to kill 50% of the population exposed. 0.5,1,2 and 4 are different concentrations in % treated for the study

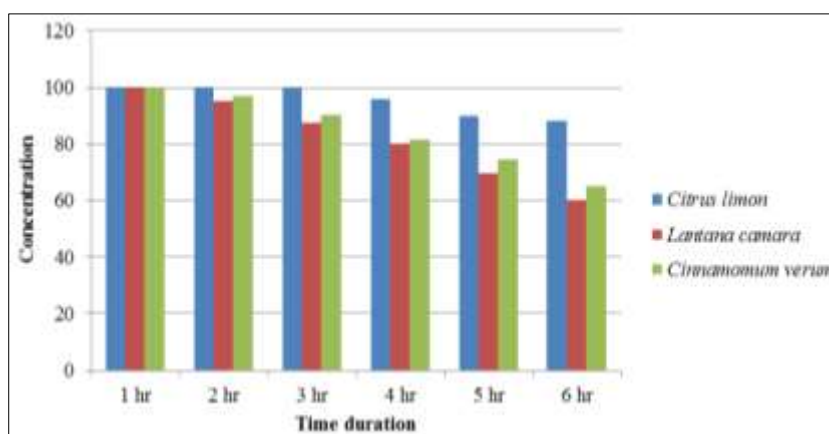


Fig 2: Adult repellency test using 10% volatile oils for 1–6 hours of application

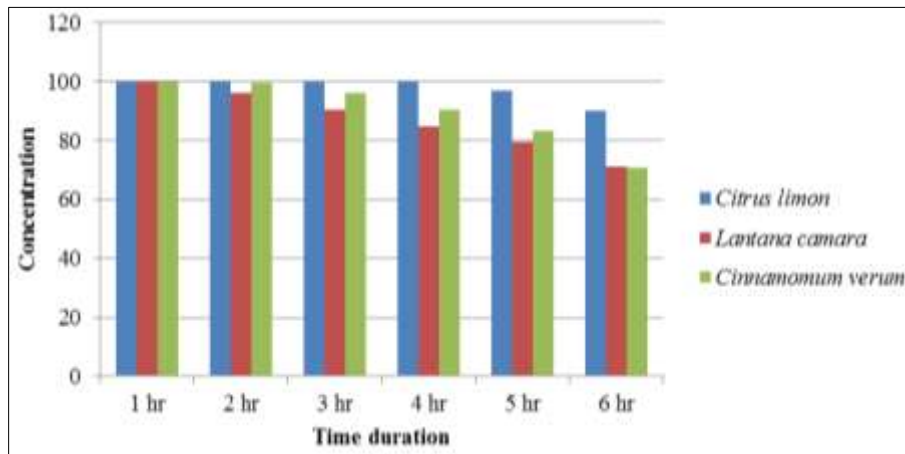


Fig 3: Adult repellency test using 25% volatile oils for 1–6 hours of application

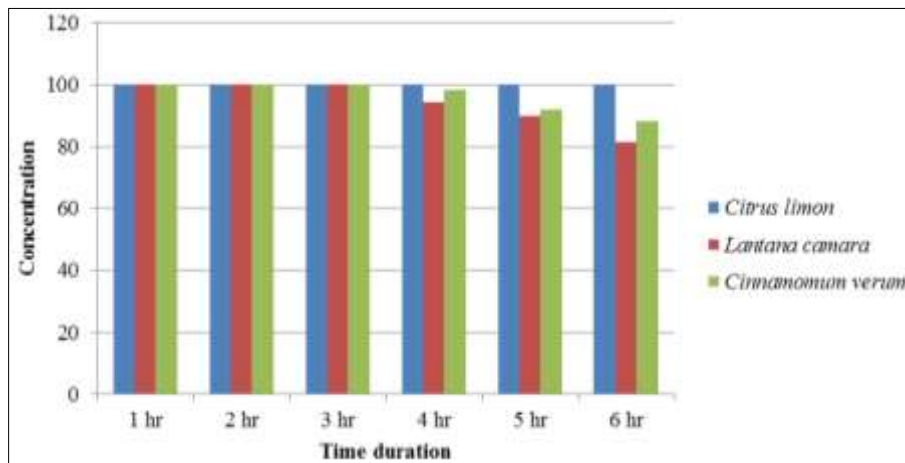


Fig 4: Adult repellency test using 50% volatile oils for 1–6 hours of application

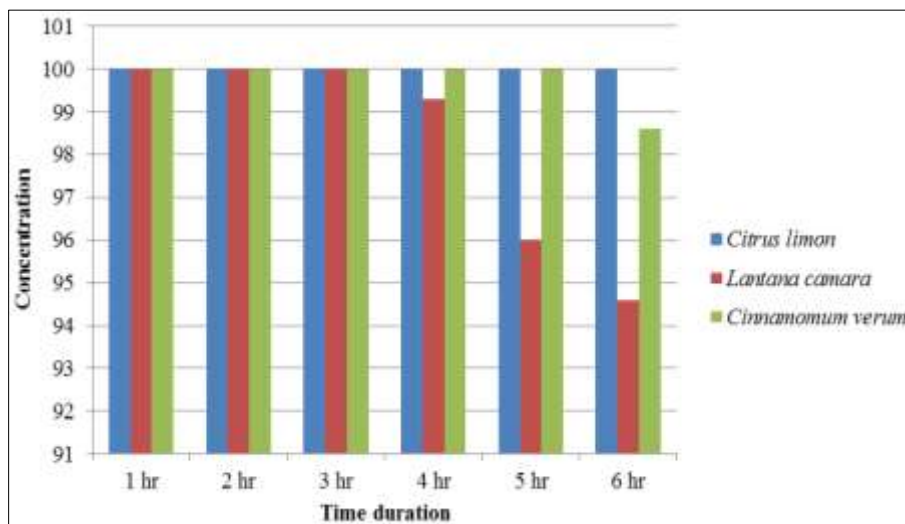


Fig 5: Adult repellency test using 70% volatile oils for 1–6 hours of application

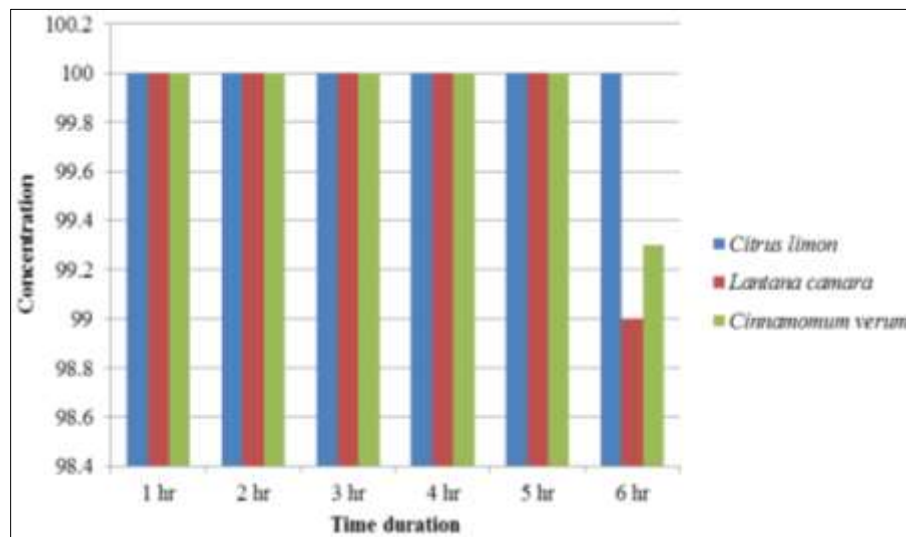


Fig 6: Adult repellency test using 100% volatile oils for 1–6 hours of application

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