



Pal Mahesh * and Chandrashekhar K.

Phytochemistry Division, National Botanical Research Institute (CSIR), Rana Pratap Marg,
Lucknow 226001. U.P. India

Abstract

Essential oil of *Piper betel* provided better protection from biting of mosquitoes *Anopheles stephensi* and *Culex fatigans* compared to known mosquito repellent citronella oil. *Piper betel* oil provided more than 4 hrs protection against *Anopheles stephensi* and *Culex fatigans* when applied at the rate of 20 μ l/cm² where as citronella oil provided only 2.2 and 2.6 hrs protection respectively. *Piper betel* oil showed more fumigant action than that of citronella oil, LC50 (30 min) was 24.81 and 20. 51 μ l/liter air against *Anopheles stephensi* and *Culex fatigans* where as LC50 of citronella oil was 32.71 and 31.97 μ l/liter air, respectively.

Keywords: *Piper betel*; Mosquito; Repellency; *Anopheles stephensi*; *Culex fatigans*; Citronella.

Introduction

Betelvine (*Piper betel*) is a perennial climber cultivated commercially in South-East Asia for its leaves. Leaves chewed in India, Malaysia and Indonesia along with other plant materials like areca nut (*Areca catechu*) and hydrated lime for mouth-refreshing, digestive and aphrodisiac properties. Leaves are put to number of medicinal uses in India. Essential oil from leaves is known for its antiseptic properties. Oil is also good for respiratory system and utilized in the treatment of bronchitis, cough and cold and chills¹⁻². Oil is also recommended as a counter irritant in swellings, bruises, painful sores and enlarged glands.

Mosquito menace is ubiquitous problem throughout the world. Large numbers of diseases are known to spread through mosquitoes. There are many attempts to control the mosquito menace and to eradicate the scourge of malaria and other severe diseases. Most of the mosquito repellent formulations available in the market are mainly prepared with active ingredients of synthetic origin. Long term exposure of newborn babies and children to parathyroid based mosquito repellents is known to cause clinical, biochemical and neurological changes⁶. There are very few natural or herbal repellants that are generally based on volatile plant oils. Most of these oils give short lasting protection, usually less than 2 hours. There is need to develop an effective and safe mosquito repellent from herbal sources. Hence, essential oil of *Piper betel* was evaluated in the laboratory for effectiveness against mosquitoes.

Material and methods³⁻⁶

Extraction of essential oil

Fresh leaves of betelvine were collected from garden of National Botanical Research Institute, Lucknow and essential oil was extracted by Hydro-distillation using Clevenger apparatus.

Repellency tests

Repellency of betel vine and citronella oil was compared using human subjects by exposing treated hands to caged mosquitoes by following method of Shreck and McGovern (1989)⁵. Hands treated with different concentrations of compound were exposed to cages containing 100 Adult female mosquitoes of *Anopheles stephensi* and *Culex fatigans* (4 day old and starved for 6 hours) for 3 minutes. Each concentration was tested against three volunteers.

*** Corresponding Author:**

Ph. No. 91-522-2297912, E-mail: drmpal@rediffmail.com

$$\text{Repellency (\%)} = \frac{\text{No. bites on control arm} - \text{No. of bites on treated arm}}{\text{No. bites on control arm}} \times 100$$

Protection time

Protection time was calculated by inserting treated hands in to the cages at 15 minutes intervals and observing for 3 minutes. The time between application of repellent and landing of at least two mosquitoes followed by confirmatory bite is taken as protection time.

Test for mortality

The toxicity of the compound was tested against the 3 days old adult females of *An. stephensi* and *C. fatigans* using airtight containers. Mosquitoes were released inside the containers and the filter paper impregnated with test compound was attached to the inner side of cap and thin layer of cotton was used to avoid direct contact and cap was immediately closed. The mortality of mosquitoes was observed after one hour.

Results and Conclusion

Both *P. betel* and citronella oil showed 100% repellency against *An. stephensi* and *C. fatigans* when applied at rate of 10 $\mu\text{l}/\text{cm}^2$. However, at low concentration piper betel oil exhibited more repellency against both the mosquito sp. than that of citronella oil. *P. betel* oil provided more than 4 hrs protection against *An. stephensi* and *C. fatigans* when applied at the rate of 20 $\mu\text{l}/\text{cm}^2$ where as citronella oil provided only 2.2 and 2.6 hrs protection respectively. *P. betel* oil showed more fumigant action against than that of citronella oil the 30 minutes LC50 was 24.81 and 20. 51 $\mu\text{l}/\text{liter}$ air against *An. stephensi* and *C. fatigans*, whereas LC50 of citronella oil was 32.71 and 31.97 $\mu\text{l}/\text{liter}$ air, respectively.

Many natural products of botanicals especially essential oils have been shown to posses satisfactory repellency effects against insects⁷. LC50 (30 min) of citronella oil was observed as 32.71 and 31.97 $\mu\text{l}/\text{liter}$ air, respectively against *An. stephensi* and *C. fatigans*.³ reported KD50 of smoke of *Cymbopogon nardus* as 45.02 min against *Ades aegypti*. Essential oil of *Piper betel* contains compounds like eugenol, methyleugenol, terpinyl acetate and caryophyllene. Organic phenolic compound extracted from *P. betel* is used for eradicating, repelling or preventing infestations of pests such as insects, mites, fungus, or parasites (US patent 6,844,369). The *P. betel* oil not only has good mosquito repellent/ mosquitocidal activity but also well for respiratory system¹⁻². Hence, it may be formulated singly or in combinations of other effective plants as mosquito repellent products in form of spray, cream, liquidator, coil, candle and sticks using suitable carries/solvents/ diluents to get better protection from mosquito bites. Such formulations help in reducing the harmful effects of synthetic mosquito repellent on human health^{4,7}.

Table 1. Repellency of piper betel oil against *Anopheles stephensi* and *Culex fatigans*
% Repellency (mean \pm S.E.) **Protection time**

| Conc. $\mu\text{l}/\text{cm}^2$ | | <i>An. stephensi</i> | <i>C. fatigans</i> | <i>An. stephensi</i> | <i>C. fatigans</i> |
|------------------------------------|--------------------|----------------------|--------------------|----------------------|--------------------|
| 2 | <i>Piper betel</i> | 18.33 \pm 1.67 | 30.00 \pm 5.0 | >1 min | >1 min |
| | Citronella | 14.00 \pm 1.0 | 16.67 \pm 1.67 | >1 min | >1 min |
| 4 | <i>Piper betel</i> | 70.00 \pm 5.77 | 91.67 \pm 8.33 | >1 min | 8.33 min |
| | Citronella | 56.66 \pm 6.67 | 86.66 \pm 6.67 | 30 sec | 5.8 min |
| 10 | <i>Piper betel</i> | 100 \pm 0.0 | 100 \pm 0.0 | 21.66 min | 18.33 min |
| | Citronella | 100 \pm 0.0 | 100 \pm 0.0 | 15.8 min | 15 min |
| 20 | <i>Piper betel</i> | 100 \pm 0.0 | 100 \pm 0.0 | >4 hrs | >4 hrs |
| | Citronella | 100 \pm 0.0 | 100 \pm 0.0 | 2.6 hrs | 2.6 hrs |

Table 2. Toxicity of Piper betel oil against *Anopheles stephensi* and *Culex fatigans*

| Dose (μ l/liter air) | <i>Anopheles stephensi</i> | | <i>Culex fatigans</i> | |
|------------------------------|----------------------------|---------------|-----------------------|---------------|
| | Citronella | Piper betel | Citronella | Piper betel |
| 5 | 10 | 0 | 6.36 | 0 |
| 12.5 | 26.6 | 25 | 23.3 | 40 |
| 25 | 50 | 50 | 53.3 | 50 |
| 50 | 60 | 70 | 60 | 82.5 |
| 75 | 66.6 | 100 | 70 | 100 |
| Control | 0 | 0 | 0 | 0 |
| LC50 | 32.71 | 24.81 | 31.97 | 20.51 |
| μ l/liter air | (27.14-40.39) | (22.25-27.58) | (27.17-38.29) | (18.35-22.84) |

Values are parenthesis fiducially limits at 95% confidence interval

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