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Bioefficacy of *Mentha piperita* essential oil against dengue fever mosquito *Aedes aegypti* L

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ABSTRACT

Objective: To assess the larvicidal and repellent potential of the essential oil extracted from the leaves of peppermint plant, *Mentha piperita* (*M. piperita*) against the larval and adult stages of *Aedes aegypti* (*Ae. Aegypti*). **Methods:** The larvicidal potential of peppermint oil was evaluated against early fourth instar larvae of *Ae. aegypti* using WHO protocol. The mortality counts were made after 24 and 48 h, and LC_{50} and LC_{90} values were calculated. The efficacy of peppermint oil as mosquito repellent was assessed using the human-bait technique. The measured area of one arm of a human volunteer was applied with the oil and the other arm was applied with ethanol. The mosquito bites on both the arms were recorded for 3 min after every 15 min. The experiment continued for 3 h and the percent protection was calculated. **Results:** The essential oil extracted from *M. piperita* possessed excellent larvicidal efficiency against dengue vector. The bioassays showed an LC_{50} and LC_{90} value of 111.9 and 295.18 ppm, respectively after 24 h of exposure. The toxicity of the oil increased 11.8% when the larvae were exposed to the oil for 48 h. The remarkable repellent properties of *M. piperita* essential oil were established against adults *Ae. aegypti*. The application of oil resulted in 100% protection till 150 min. After next 30 min, only 1-2 bites were recorded as compared with 8-9 bites on the control arm. **Conclusions:** The peppermint essential oil is proved to be efficient larvicide and repellent against dengue vector. Further studies are needed to identify the possible role of oil as adulticide, oviposition deterrent and ovicidal agent. The isolation of active ingredient from the oil could help in formulating strategies for mosquito control.

1. Introduction

Mosquitoes are a serious threat to public health transmitting several dangerous diseases for over 2 billion people in the tropics[1]. *Aedes aegypti* (*Ae. aegypti*), the primary carrier for viruses that cause dengue fever, dengue hemorrhagic fever and yellow fever is widespread over large areas of the tropics and subtropics. Mosquito control and personal protection from mosquito bites are currently the most important measures to control these diseases. Many approaches have been developed and tried to tackle mosquito menace. The use of larvicides and repellents is an obvious practicality and economical means of preventing the transmission of these diseases to humans.

The common approach for the control of mosquito vectors and reducing the transmission of human pathogens is based on the chemical insecticide-based intervention measures[2]. However, in the past, the frequent and repeated use of chemical insecticides has resulted in the worldwide development of insecticide resistance, destabilisation of the ecosystem and toxic effects on human beings and non-target organisms[3]. Thus, there is an urgent need to develop new insecticides for controlling mosquitoes which are more environmentally safe, biodegradable and target-specific against the mosquitoes. In recent years, much effort has, therefore, been focused on plant extracts or phytochemicals as potential sources of mosquito control agents or as lead compounds[4,5].

Many researchers have reported the effectiveness of plant extracts or essential oils as efficient mosquito larvicides and repellents without posing hazards of toxicity to humans[6-10]. Several extracts and compounds from different plant families have been evaluated to show new and promising larvicides[11-13]. Further, repellency is known to play an important role in preventing the vector borne diseases by reducing man-vector contact. Ethno-botanical studies

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show that in some village communities, the use of plant repellents to reduce human vector contact is a common practice[14].

Essential oils are natural volatile substances found in a variety of plants. Commercially, essential oils are used in four primary ways: as pharmaceuticals, as flavor enhancers in many food products, as odorants in fragrances, and as insecticides. The plant oils have received much attention as potentially useful bioactive compounds against insects showing a broad spectrum of activity, low mammalian toxicity and degrading rapidly in the environment. Peppermint oil extracted by steam distillation from the leaves of *Mentha piperita* (*M. piperita*) has a long tradition of medicinal use. It has high menthol content, and is often used in certain food items. It has also been reported that peppermint oil reduces colic abdominal pain and causes a major reduction in irritable bowel syndrome symptoms[15]. The oil is also reported to contain menthone and menthyl esters. Menthone, present in high concentration in peppermint oil, is reported to act as a natural pesticide[16]. Still, the insecticidal properties of peppermint oil have not been explored. Keeping in view the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, the availability, low budget and less environmental impact, this study was undertaken to assess the larvicidal and repellent potential of the essential oil of peppermint plant, *M. piperita* against larval and adult stages of *Ae. aegypti*. The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive chemical compounds from indigenous plant source.

2. Materials and methods

2.1. Mosquito rearing

The present investigations employ the dengue fever mosquito, *Ae. aegypti*, originated from fields of Delhi and surrounding areas. The colony was maintained in an insectary at (28±1) ℃, (80±5)% RH and 14:10 L/D photoperiod[17]. Adults were supplied with freshly soaked deseeded raisins. Periodic blood meals were provided to female mosquitoes for egg maturation by keeping restrained albino rats in the cages. The eggs were collected in a bowl lined with Whatman filter paper and were allowed to hatch in trays filled with de–chlorinated water. Larvae were fed upon a mixture of yeast powder and grinded dog biscuits. The pupae formed were collected and transferred to the cloth cages for adult emergence.

2.2. Larvicidal bioassay

The larvicidal bioassay was performed at (28±1) ℃ on the *Ae. aegypti* larvae in accordance with the WHO method for mosquito larvae with slight modifications. The graded series of peppermint oil was prepared using ethanol as the solvent. The early fourth instar larvae of *Ae. aegypti*, in batches of 15, were taken in plastic bowls containing 99 mL of distilled water and transferred to glass jar containing 100 mL of distilled water and 1 mL of the particular concentration of oil. Four replicates were carried out simultaneously for each dilution making a total of 100 larvae for each concentration. Controls were exposed to the solvent, *i.e.* ethanol alone.

During the treatment period, no food was offered to the larvae. The larvae were considered moribund if, at the end of 24 h, they showed no sign of swimming movements even after gentle touching with a glass rod. The dead and moribund larvae were recorded after 24 h as larval mortality. The whole set up was kept undisturbed for another 24 h and mortality counts were recorded again after 48 h.

2.3. Statistical analysis of data

The larvicidal tests with more than 20% mortality in controls and pupae formed were discarded and repeated again. If the control mortality ranged between 5%–20%, it was corrected using Abbott's formula.

$$\text{Corrected mortality} = \frac{\%\text{ Test mortality } - \%\text{ Control mortality}}{100 - \%\text{ Control mortality}} \times 100$$

The data were subjected to regression analysis using computerized SPSS 11.5 Programme. The LC_{50} and LC_{90} values with 95% fiducial limits were calculated in each bioassay to measure difference between the test samples. The results obtained with different oils were analyzed using Student's *t*–test with statistical significance considered for $P \leqslant 0.05$.

2.4. Adult repellency bioassays

The repellency of the peppermint oil against dengue vectors was evaluated using the human–bait technique. For investigation, 25 laboratory–reared, blood–starved, adult female *Ae. aegypti* that were between 3 and 10 days old were placed into separate laboratory cages (45 cm×45 cm×40 cm). Before each test, the forearm and hand of a human subject were washed with unscented neutral soap, thoroughly rinsed, and allowed to dry 10 min before extract application. An area of (5 cm×5 cm) on each forearm of five human volunteers was marked out with a permanent marker. Approximately 0.1 mL of the oil was applied to the marked area of one forearm of each volunteer while the other forearm was treated with ethanol as control. During the test, the forearm was covered by a paper sleeve leaving the marked area open. The control and treated arms were introduced simultaneously into the cage. An attempt of the mosquito to insert its stylets was considered a bite. The numbers of bites were counted over 3 min, every 15 min, from 1 100 h to 1 400 h.

Protection time was recorded as the time elapsed between repellent application and the observation period immediately preceding that in which a confirmed bite was obtained. If no bites were confirmed at 180 min, tests were discontinued, and protection time was recorded as 180 min. In case, if during the observation period no mosquito landed on the control arm or attempted to bite, the trial was discarded, and the test was repeated with a new batch of mosquitoes to ensure that lack of bites was due to repellence and not because mosquitoes are not predisposed to get a blood meal at the time. The experiments were conducted three times in separate cages, and in each replicate, different volunteers were used to nullify any effect of skin differences on repellency.

The percentage protection was calculated by using the following formula:

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

3. Results

The investigations revealed that when the early fourth instars of *Ae. aegypti* were exposed to peppermint oil for 24 h, the LC_{50} and LC_{90} values obtained were 111.9 and 295.18 ppm, respectively. The essential oil was proved to be 11.8 % more effective when the exposure of the larvae to essential oil continued for 48 h, exhibiting an LC_{50} value of 98.66 ppm.

The results obtained proved and established the efficacy of the *M. piperita* essential oil against the dengue vector larvae. All the treatments resulted in complete mortality of larvae without any pupal or adult emergence. The control or untreated groups did not show any mortality within 48 h. The larvae developed into pupae and then adults within 60–72 h.

The essential oil of *M. piperita* has exerted promising and remarkable repellent activity against *Ae. aegypti* adults. The present studies revealed the percentage protection in relation to time (min). Our investigations resulted in 150 min of complete protection time with 0.1 mL of oil applied on the arms of human volunteers. After 150 min, the efficacy of oil decreased a little resulting in non–repellency of 7% of mosquitoes. Consequently, the % protection decreased to 27% in half an hour. First bite of the dengue vector was observed after 165 min of oil application, making the % repellency and protection of 96% and 88.8%, which decreased to be 93.2% and 73.1%, respectively after 180 min. The control treatment did not provide any protection even during the first trial. The number of bites in control treatment ranged only from 3 to 9 in 3 min of exposure period spread over 180 min.

4. Discussion

Essential oils extracted from different plants have been reported to have larvicidal and repellent properties against *Ae. aegypti*[7,18–20]. Though few reports are available regarding the potential of *M. piperita* oil as repellent against insects, no work has been carried out regarding its potential as mosquito larvicide agent. The present investigations were performed to assess the potential of essential *M. piperita* oil as larvicidal and repellent agent against *Ae. aegypti*.

Our investigations clearly showed that essential oil extracted from *M. piperita* possesses excellent larvicidal efficiency against dengue vector. The bioassays performed resulted in an LC_{50} value of 111.9 ppm and LC_{90} value of 295.18 ppm after 24 h of exposure. It was also observed that the toxicity of the oil increased when the larvae were exposed to the oil for longer duration. Though there are no reports available regarding the potential of peppermint oil as mosquito larvicide, several reports are available on other oils which reveal their efficacy against mosquito larvae. Cheng *et al*[21] compared the essential oils from the leaves of eight provenances of indigenous cinnamon (*Cinnamomum osmophloeum* Kaneh.) and reported that the essential oils of cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type had an excellent inhibitory effect against the fourth–instar larvae of *Ae. aegypti* with LC_{50} values of 36 mg/L and 44 mg/L; and LC_{90} values of 79 mg/L and 85 mg/L, respectively. The larvicidal activity of cinnamon and other oils were recorded by Zhu *et al*[22,23] against 4th instars of *Aedes albopictus*, *Ae. aegypti*, and *Culex pipiens* pallens. Amer and Mehlhorn[7] reported the larvicidal properties of *Lippia citriodora* against *Ae. aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. Senthilkumar *et al*[24] reported larvicidal effect of *Blumea mollis* essential oil against *Culex quinquefasciatus*, with LC_{50} and LC_{90} values of 52.2 and 108.7 mg/L, respectively.

According to Gleiser and Zygadlo[25], the essential oils of *Lippia turbinata* and *Lippia polystachya* exhibit LC_{50} values of 74.9 and 121 mg/L, respectively against *Culex quinquefasciatus*. The essential oil of *Zanthoxylum armatum* was tested against three species of mosquitoes by Tiwari *et al*[26]. He found that among all the three species *Culex quinquefasciatus* was the most sensitive with LC_{50} and LC_{95} values of 49 and 146 ppm, respectively followed by *Ae. aegypti* and *Anopheles stephensi* with LC_{50} values in the range of 54–58 ppm.

Our investigations also revealed the excellent repellent properties of *M. piperita* essential oil against adults *Ae. aegypti* resulting in 100% protection till 150 min. After next 30 min, only 1–2 bites were recorded as compared with 8–9 bites on the control arm. In 2009, Nour *et al*[27] reported that the essential oils from four basil accessions, *Ocimum basilicum*, conferred complete repellency against *Anopheles mosquito* lasting for 1.5 to 2.5 h per one application of 0.1 mL to a human volunteer's arm. The essential oil of *Zingiber officinalis* showed repellent activity at 4.0 mg/cm^2 and provided 100% protection up to 120 min against *Culex quinquefasciatus*[28]. *Trachyspermum ammi* seed oil could achieve a repellency of 45.0% with repellent dose (RD_{50}) observed as 25.02 mg/mat against *Anopheles stephensi* adults[29]. Earlier, Tawatsin *et al*[30] have reported that the essential oils extracted from 18 plant species, belonging to 11 families were more effective against the night–biting mosquitoes (*Anopheles dirus, Anopheles albopictus* and *Culex quinquefasciatus*) exhibiting repellency for 4.5–8 h than against *Ae. aegypti* (repellency 0.3–2.8 h). Essential oil of *Cinnamomum zeylanicum, Zingiber officinale*, and *Rosmarinus officinalis* also showed repellent activities against *Anopheles stephensi*, *Ae. aegypti*, and *Culex quinquefasciatus*[31].

There are reports available where essential oils have shown repellent properties in the fields. The essential oils extracted from some Verbenaceae plants have shown repellent and/or insecticidal effects against mosquitoes[8,32]. Petroleum ether extracts of *Vitex agnus* castus leaves offered 8 h of bite protection (2 mg/cm^2) by different mosquito species in the field[33,34]. Rural populations of Tamil Nadu, India burn leaves of this plant with grass as fumigant against mosquitoes. *Lippia citriodora* was reported to have repellent properties against *Ae. aegypti*[32] and *Culex quinquefasciatus*[8] and larvicide against *Ae. aegypti*, *Anopheles stephensi,* and *Culex quinquefasciatus*[7].

In conclusion, an attempt has been made to evaluate the possible role of peppermint essential oil to control mosquitoes. As natural products are generally preferred in vector control measures due to their less deleterious effect on non–target organisms, their innate biodegradability and keeping in view the resistance developed by the mosquito larvae against chemical insecticides, it is worthwhile to identify new active compounds from natural products against mosquitoes. Hence, the results reported here open the possibility of further investigations of efficacy of peppermint oil in terms of its adulticidal, oviposition deterrent and ovicidal action against mosquitoes.

Conflict of interest statement

We declare that we have no conflict of interest.

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