


REPELLENCY OF AROMATIC PLANTS BASED INGREDIENTS AGAINST MOSQUITOES

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ABSTRACT: Mosquitoes are the most important single group of insects and transmitting several harmful diseases such as malaria, filaria, dengue, yellow fever and viral encephalitis. Controlling of these diseases by the application of easily degradable plant compounds is considered to be one of the safest methods to mosquito bite prevention and it's as an alternative source for the synthetic pesticides. Therefore, a study was made to monitor the effect of plant ingredients (*Aloe vera* and *Allium sativum*) on different species of mosquitoes. *Aloe vera* gel and garlic bulb were dried under the shade and the powdered materials were extracted in methanol and further partially purified using different organic solvents. Active ingredients of both plant extracts were mixed with inert chemical magnesium silicate as base materials. An active ingredient were tested at different concentrations of 0.125%, 0.25%, 0.5%, 1%, 2% against adult female mosquitoes, *Culex*, *Anopheles* and *Aedes* (3 - 5 days old) in a laboratory conditions with different intervals. The results suggested that at high concentration of active ingredients showed greater repellent activity in longer time. Even though, low concentration of active ingredients showed greater repellent activity at short time without any side effect.

Key Words: Repellency, *Aloe vera*, *Allium sativum*, *Culex*, *Anopheles* and *Aedes* vectors

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INTRODUCTION

Mosquitoes are the most important blood sucking Arthropods, capable of transmitting pathogens of many diseases like malaria, yellow fever, dengue fever, filariasis, Chikungunya and viral encephalitis causing serious health problems to humans. Many millions of people all over the world, particularly in South and Southeast Asian countries suffer from mosquito-borne diseases. In view of this, mosquito control is an important step towards the prevention of such harmful infections. One of the effective methods to control these diseases has been to target the vectors for interrupting the transmission. Personal protection is also one of the established methods to prevent mosquito bites. Use of household mosquito repellents by individuals and communities also play an important role particularly against day-time biting mosquito species such as *Aedes*, the vector of dengue, chikungunya and *Anopheles*, the vector of malaria (Mittal *et al.*, 2011).

Therefore, the efficient way to prevent these diseases is to control mosquito vector populations and prevent mosquito bites. Many consumers, reluctant to apply DEET (N,N-diethyl benzamide) to skin, deliberately seek out other repellent products (Adeniran *et al.*, 2012). Although there are a number of effective mosquito repellents containing synthetic chemicals. Such as, DEET (Coleman *et al.*, 1993), KBR 3025 (Yap *et al.*, 1998) and IR 3535 (Thavara *et al.*, 2001) which are currently available in the market, there is increasing concern with regard to their toxicity. Synthetic chemicals are generally been regarded as safe, but toxic effects have been recorded, including encephalopathy in children, urticarial syndrome, anaphylaxis, hypotension and decreased heart rate (Peterson *et al.*, 2001). Because of this fact, researchers are seeking natural ways to repel mosquitoes (Lawal *et al.*, 2012). Usually, plant derived repellents do not pose hazards of toxicity to humans and domestic animals and are easily biodegraded. Many plant compounds have a pleasant fragrance, relatively low mammalian toxicity, and a vapour pressure almost ideal for action as a volatile spatial repellent. Essential oils as mosquito repellents indicates that citronella oils from *Cymbopogon nardus* and *C. winterianus* have been reported to be the most widely used insect repellents (Soonwera, 2015). In view of fact that the present study was undertaken to prevent man-mosquito contact by using some aromatic plants based natural repellents instead of commercially available electronic liquidator, mosquito coil and skin lotion.

MATERIALS AND METHODS

Plant extraction and repellency cream preparation

Aloe vera L. (Family: Xanthorrhoeaceae) gel from leaves and *Allium sativum* L. (Family: Amaryllidaceae) bulbs were collected and purchased from Srivilliputhur Taluk, India and identified with voucher specimen. The plant powdered materials were weighed and extracted overnight in analytical grade methanol (MeOH) in the ratio 1:10 W/V over a magnetic stirrer. The MeOH extract was filtered and concentrated in a vacuum evaporator at 45°C under low pressure and the residues of the extracts were defatted by washing them thrice with equal volume of petroleum ether (PE) in a separating funnel. The two extract were collected separately and concentrated to dryness. The residue of the defatted MeOH extract was dissolved in ethyl acetate. The ethyl acetate soluble and ethyl acetate (EtoAc) insoluble extract were separated. The EtoAc soluble extracts were washed with equal volume of double distilled water. The residues obtained at each stage of the purification process were weighed and the yield in relation to the weight of the starting material was calculated. Further tests were performed with the most active ingredients only. Partially purified ingredients were dried by lyophilization and different concentrations of all the residues were prepared with base, magnesium silicate. Efficiency of each stage of ingredients was tested for their repellency protection against female adult mosquitoes, *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*.

Evaluation of repellency under laboratory conditions

The test repellents were evaluated for repellency under laboratory conditions using human-bait method (Tawatsin *et al.*, 2001; Thavara *et al.*, 2001). The test period was up to 11 hours, depending on duration of repellency of each repellent. The tested mosquito species were *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* and these mosquitoes were uninfected laboratory strains and were reared for over 10 generations. The repellency assay was performed in a dark room with red light as the only source of illumination (WHO, 1996). The room temperature and relative humidity were controlled at $28 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ respectively to mimic the feeding conditions for female mosquitoes. Cages (60 × 60 × 60 cm) made of aluminium sheet at the bottom, mosquito net screen on sides and top, and a cotton stockinet sleeve for access on the front, were used in the dose response assays. Different concentrations (0.125 - 2% w/w) of selected active ingredients and blends of these were prepared by adding 200mg of each plant ingredient in 10 gm of analytical magnesium silicate, followed by successive two-fold additions with magnesium silicate to obtain the other concentrations.

Before applying the ingredients, the volunteers had no contact with any lotion, perfume, oil, or perfumed soap on the day of the bioassay. The forearm (average area of 697 cm^2) of each volunteer from the elbow to the hand was washed with water and left to dry. The test sample (0.125%) was spread as evenly as possible on one of the forearms of a volunteer from the wrist to the elbow. The rest of the hand was covered with a glove. Magnesium silicate (0.2gm) was dispensed on the other forearm to serve as control. The control and treated arms were interchanged regularly to eliminate bias. The control arm was first introduced into the cage for 10 min immediately after introduction of the mosquitoes. The number of mosquitoes that landed on the arm was recorded, and the insects were shaken off before they imbibed any blood. This was followed by exposure of a volunteer arm first to the lowest concentration (0.125% w/w) of the test sample followed by sequential exposures to progressively higher concentrations (2% w/w) of the sample, each time to fresh mosquitoes in a clean cage. The test arm of the volunteer was washed using a non-perfumed soap and tap water and allowed to dry naturally for at least 20 min before dispensing the subsequent concentration.

Field evaluation

The selected active ingredients were evaluated for repellency against wild population of mosquitoes under field conditions. Field evaluations were conducted by four volunteers (aged 25- 57 years) at a residence place located in Housing Board Colony, Sivakasi. This place was chosen to be the study site as it had developmental sites for a variety of mosquito species. The evaluations were carried out at locations with minimal wind disturbance in an opened-building, which had only roof and there were some water jars placed in the building.

Mosquitoes therefore could come from surrounding areas to feed on volunteers. Each volunteer was treated with 2% of the test repellent on one leg (from knee to ankle) whereas the other leg was left as control. The volunteers were positioned in a row, 5 m apart from each other and they caught all of the mosquitoes landing on or biting both their legs in the desired area (from knee to ankle) within a 10-minute period. The tests were carried out for 4 hours from 1800 h to 2200 h. The collected mosquitoes then were identified to species in laboratory. Repellency of each repellent was assessed through comparisons of mosquitoes collected on control (untreated) and treated legs. The reduction in biting was calculated (Yap *et al.*, 1998).

Data analysis. Percentage protective efficacy (PE) was calculated using the formula $PE = (C-T/C) \times 100\%$, where C and T are the mean numbers of mosquitoes that landed on the control and test arm, respectively (Sharma *et al.*, 1994, Matsuda *et al.*, 1996). Means were subjected to analysis of variance (ANOVA) and compared by the Student-Newman-Keuls test (SAS, 2000).

RESULTS AND DISCUSSION

Repellent activities of selected active ingredients were studied for physical characters like nature, odour, colour and consistency. Initially, the repellent activity of different partially purified extracts like methanol, petroleum ether, Ethyl acetate soluble were used against starved *Cx. quinquefasciatus* females varied according to plant extracts and the different doses used at 1, 2, 3, 4 and 5%. Of this Ethyl acetate soluble extracts induced high degree of repellency in both selected plants. Therefore, the extracts were used to further study and also this extract is called as active ingredients. Similarly, the essential oils extracted by steam distillation from leaves of five plant species *Centella asiatica* L., *Ipomoea cairica* L., *Momordica charantia* L., *Psidium guajava* L. and *Tridax procumbens* L. were evaluated for their topical repellency effects against malarial vector *An. stephensi* in mosquito cages. All essential oils were tested at three different concentrations (2, 4 and 6%). Of these, the essential oils of *I. cairica*, *M. charantia* and *T. procumbens* exhibited relatively high repellency effect (>300 minutes at 6% concentration), followed by *C. asiatica* and *P. guajava* which showed less effective (<150 minutes at 6 % concentration) (Rajkumar and Jebanesan, 2004).

The protection results on arm treated by 0.125 - 2% active ingredient of *A. vera* against three mosquito species shown in figure 1. There was no repellency with the control (Magnesium silicate alone). The protection time of *A. vera* was the longest and the percentage of protection remained 100% after an exposure of an hour. The protection percentage of triplicate assay was more than 74.2% after 11 h treatments. There were no significant differences among the repeated measures analysis of variance and also significant variance among the different time sections. The repellency of the active ingredients against *Cx. quinquefasciatus* was high during the first hour and there was no significant difference among those exposed for 3 to 11 h. There was no significant correlation between the time of interval and active ingredient of *A. vera* cream. The protection results on arm treated by 0.125 - 2% active ingredient of *A. vera* cream against *An. stephensi* over 11 h in laboratory conditions shown in figure 1. For instance, genus *Cymbopogon*, which yields the most popular repellents in the world, *C. excavates* gave 100% repellency for 2 h, when it was evaluated in the laboratory against *An. stephensi*; its repellency decreased to 59.3% after 4 h (Govere *et al.*, 2000).

The average numbers of mosquitoes landing or biting the bare hand of the volunteers (within 10 seconds before the start of each exposure) were 0.125% - 2% active ingredient, *A. sativum* cream applied on the hand against selected mosquito species. This confirms the test mosquitoes were host seeking during the test periods. There were significant differences in repellency obtained during the different exposure times for repellent, *A. sativum* against the test mosquitoes (Figure 2). The results show the repellency was directly proportional to the concentrations of active ingredient and inversely proportional to the exposure time. In other words, selected active ingredient, *A. sativum* cream was provided significantly different repellencies during the exposure periods against *Cx. quinquefasciatus*. There were no significant differences among the repeated measures analysis of variance (F= 6.34, 3 df, P = 0.009), and there was also significant variance among the different time sections (F = 6.38, 7 df, P = 0.009). The repellent activity of active ingredients for *Cx. quinquefasciatus* was higher during the first 1 hour and there was no significant difference among those exposed for 3 h to 11 h. There was no significant correlation between the time of interval and active ingredient of *A. sativum* cream (P = 0.15).

The repellency of the *A. vera* against selected mosquito species were between 0 and 11 hours is shown in Figure 3. All the *A. vera* provided significantly lower repellency than *A. sativum* against *Cx. quinquefasciatus* but higher activity for against *Ae. aegypti*. Regarding the repellency against *Ae. aegypti*, it is interesting to note that *A. vera* cream were provided excellent repellency of 11 hours, equally to commercial cream against *Ae. aegypti*.

Relative repellency (mean protection time) under laboratory conditions provided by active plant ingredient, *A. sativum* cream against the *An. stephensi* is shown in figure 4. *A. sativum* cream demonstrated higher repellency for 1 hour and 11 hours against *An. stephensi*. Mean (\pm SD) biting on the control areas (the untreated bare hands) for *Anopheles* was too higher. On the other hand, *An. stephensi* was significantly less sensitive to active ingredient with mean protection time over 11 hours. The present results of maximum 2% per whole forearm gave longer protection time. It is similar to few worker like, Yang and Ma (2005) reported that six essential oils: Asteraceae oil, Rutaceae oil, Menthapiperta oil, Carvacryl oil, Citronella oil and Eucalyptus oil were tested for evaluation of their repellent effects against *Aedes albopictus* mosquitoes under laboratory conditions.

As for the repellency results against *Cx. quinquefasciatus*, the *A. sativum* were demonstrated a relatively high degree of repellency, ranging from 1 to 11 hours, while those of *Ae. aegypti* were slightly different hours (Figure 4). Unlike the effect against *An. stephensi* excellent repellency against *Cx. quinquefasciatus* was found in *A. sativum* cream, which was statistically equal to *A. vera*. In contrast, there was no significant difference in repellency obtained for *A. vera* and *A. sativum* against the three mosquito species at the three exposure periods.

Results of field trials of *A. vera* and *A. sativum* creams during evening 6:00 to 10:00pm (four hours) bait collections against different species of mosquitoes are shown in figure 5. Percentage of protection with *A. vera* cream applied at 2 % was 100% against *An. culicifacies*, *An. stephensi*, *An. anularis*, *An. subpictus*, *Cx. quinquefasciatus*, *Cx. pipiens*, *Cx. jenseni* up to 4 h of observation time. The per cent protection with *A. sativum* cream applied at the same dosages was 100% against all the above mentioned species (Figure 5). In the field conditions, our results may be compared with the data obtained from a plant based product contained *p*-menthanediol (PMD), extracted from Lemon Eucalyptus (*Eucalyptus maculatecitriodon*) as the active ingredient. It has shown particular promise as a repellent of botanical origin in the field, at doses of 0.8 - 2.0 g/leg, 50% PMD rendered complete protection from biting for 6 - 7.75 hours (Trigg, 1996) while 3% *A.vera* and *A.sativum* in cream forms gave shorter complete protection (4 hours) against *Ae. aegypti*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Ma. uniformis*, and other nuisance mosquitoes.

The results of present study were clearly better than the efficacy test on 14% citronella cream against *Culex* mosquitoes under field conditions for only 1 hour, and showed that the cream could prevent at least 90% of mosquito attacks in 13/20 volunteers who applied enough cream (Jaruwichitratana et al., 1988). In contrast to our results, both active extracts from *A. vera* and *A. sativum* provided 100% repellency for 4 hours against wild mosquitoes in the field conditions, which was carried out using a pair of volunteers who sat together, one of whom was treated with the cream which the other was not and this work similar to Ansari and Razdan, 1994. These findings may lead to new and more effective strategies for protection from and control of mosquitoes. Therefore, the selected plants provide a remarkable repellency for medically important species like *Culex*, *Aedes* and *Anopheles*.

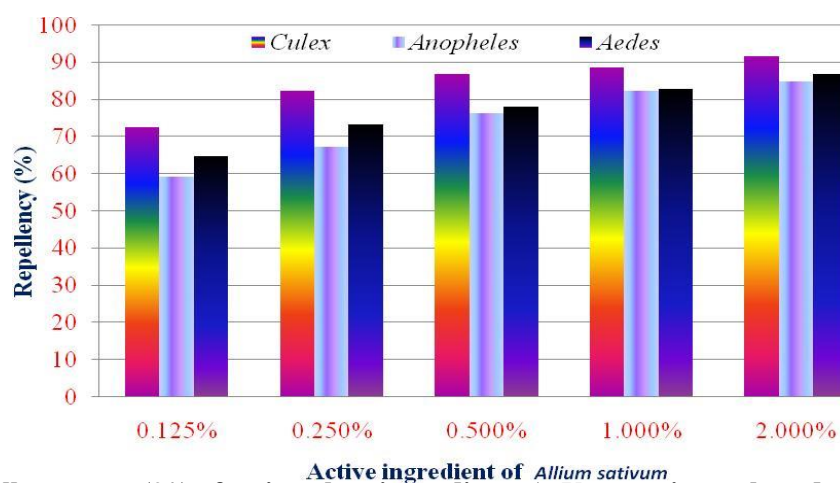


Figure 1: Mean repellency rate (%) of active plant ingredient, *A. Vera* against selected mosquito species over 11 hour exposure under laboratory conditions. Repellency obtained from a 10 minute exposure time.

However, further evaluations are required to determine chemical nature and its mode of action on mosquitoes repelled and an effective alternative for repelling biting mosquitoes in field conditions.

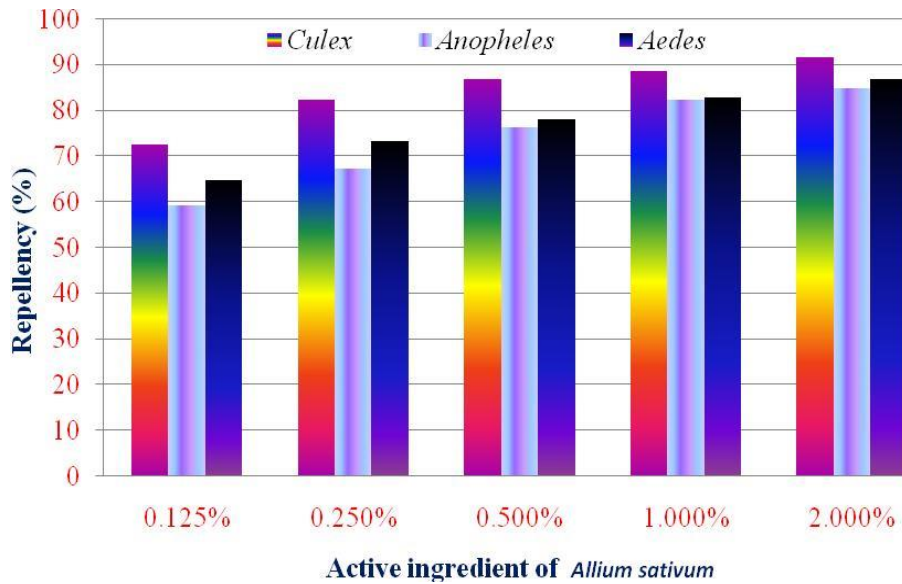


Figure 2: Mean repellency rate (%) of active plant ingredient, *A. sativum* against selected mosquito species over 11 hour exposure under laboratory conditions.

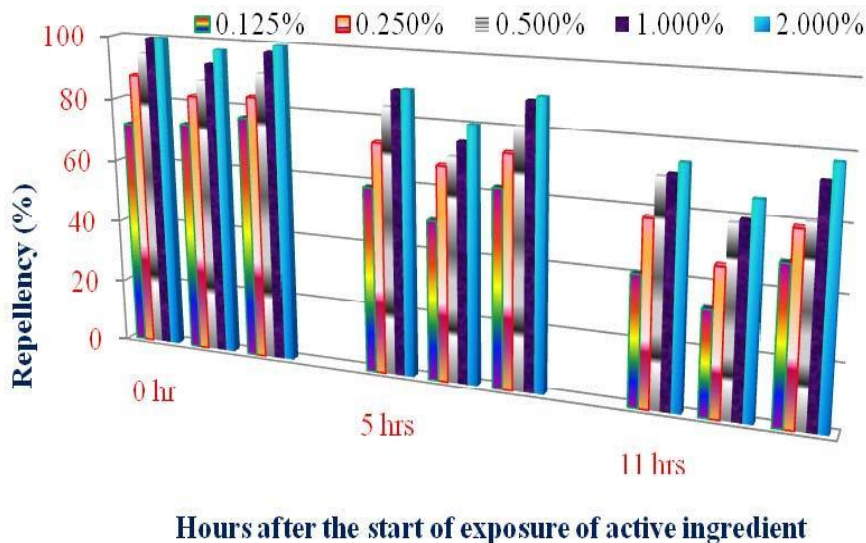


Figure 3: Percentage of repellency of active plant ingredient, *A. vera* against selected mosquito species over 11 hour comparison under laboratory conditions.

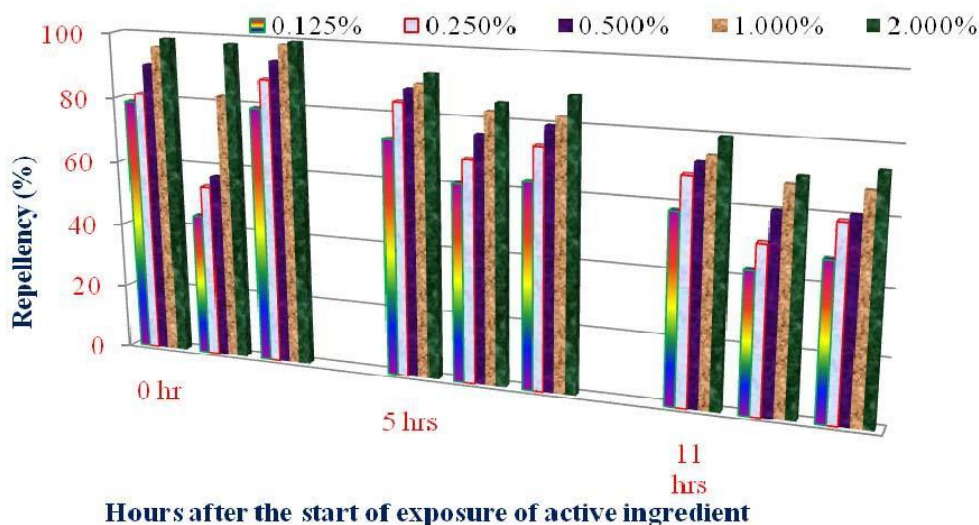


Figure 4: Percentage of repellency of active plant ingredient, *A. sativum* against selected mosquito species over 11 hour comparison under laboratory conditions.

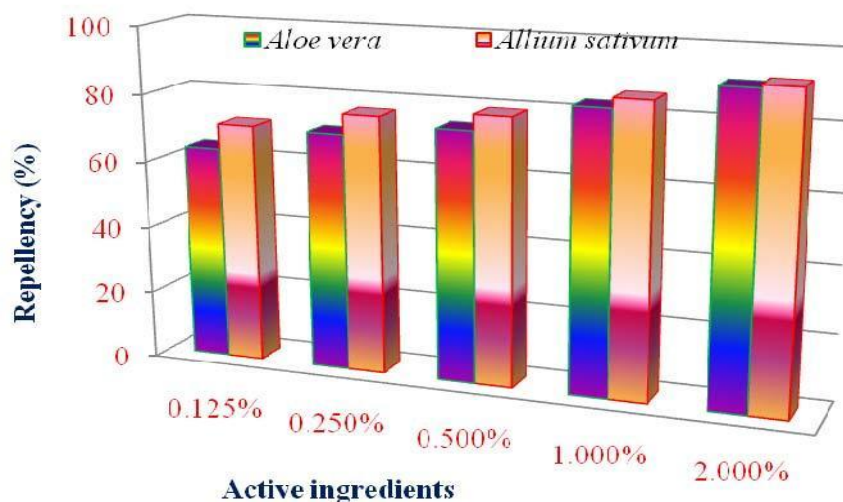


Figure 5: Mean repellency rate (%) of active plant ingredients, *A. vera* and *A. sativum* against mosquitoes over 11 hour exposure under field conditions.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

ACKNOWLEDGEMENT

We profoundly thank UGC, New Delhi for providing financial support to carry out this work under MRP Scheme. We also thank the Management and Principal, Ayya Nadar Janaki Ammal College, Sivakasi for provided great support by making the availability of various institutional facilities.

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ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY



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