**Advances in Bioresearch**
Adv. Biores., Vol 5 (1) March 2014: 50-55
©2014 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV 7.20 [Poland]

Advances in Bioresearch

## ORIGINAL ARTICLE

# Mosquito Repellent activity of *Syzygium aromaticum* (clove) against Malaria Vector, *Anopheles stephensi*

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**ABSTRACT**
*Repellent potential of acetone, hexane and ethyl acetate extracts of Syzygium aromaticum (clove) was screened under laboratory conditions against malaria vector Anopheles stephensi Liston (Diptera: Culicidae) and compared with the DEET (N, N Diethyl 1-3 methyl Benzamide, formerly known as diethyl 1-m-toluamide). Results obtained from the laboratory experiments showed that the hexane extract of clove was more effective for repellency of malaria vector, Anopheles stephensi even at 2.5, 5 and 10% concentrations. Clear dose response relationships were established with the highest dose of 5% clove extract evoking 100% repellency. Percent protection obtained against An. stephensi was 100% repellency in 6 hours. DEET- 2.5% showed 100% repellency in 6 hours against the target organism. The extract can be applied as an effective personal protective measure against mosquito bites.*
***Keywords:*** *Syzygium aromaticum, repellent, Plant extract, DEET, Mosquitoes*

*Received 09/01/2014 Accepted 02/03/2014*


**How to cite this article:**
S.P.Singh and Lalit M.Mosquito Repellent activity of *Syzygium aromaticum* (clove) against Malaria Vector, *Anopheles stephensi***.** Adv. Biores., Vol 5 [1] March 2014: 50-55**.** DOI: 10.15515/abr.0976-4585.5.50-55

## INTRODUCTION

Malaria contributes the major disease burden and its control is hampered many operational and technical reasons and among the technical reason insecticidal resistance, namely, development of insecticide resistance in malaria vectors to the commonly used synthetic chemical insecticides in public health sprays has made the disease control more difficult [1]. Mosquito population has not been reduced significantly after continuous use of synthetic insecticides and Anophelines retain their potential to support the outbreak and development of malaria in endemic areas. *Anopheles stephensi* Liston and *Anopheles culicifacies* Giles are two major vectors of malaria in India and later is a sibling species complex. There are five sibling species present named A, B, C, D, and E, of which species A, C, D, and E are vectors while B is a poor vector [2].*Culex quinquefasciatus* Say transmits filariasis while dengue fever and DHF are transmitted by the vector *Aedes aegypti* Linn.

Repellents can be used by individuals for personal protection and thus help in prevention of the disease transmission [3]. They are also the primary means of mosquito-borne disease prevention available in areas where vector control is not practical [4-5]. The majority of commercial repellent products contain the DEET (Diethyl 1-3 methyl Benzamide, formerly known as diethyl 1-m toluamide), which was first Synthesized in 1954[6]. One promising new repellent is the piperidine compound, A13-37220, which provides equal protection against certain mosquitoes than that obtained with DEET [7-9]. Despite the significant repellency of A13-37220, there are concerns over its safety.

Many botanical substances, *Mentha piperita* , *Eucalyptus maculate citriodon* , *Pelargonium citrosum* , *Azadirachta indica* and *Lantana camara* [10-14], have been reported as being repellent against adult mosquitoes. Some promising essential oils such as citronella, lemon, eucalyptus, neem, and peppermint oils are derived from these plants and they are currently available in several commercially formulated

repellents. However, their repellency is still lower in both efficacy and duration than that in currently used chemicals such as Deet and A13-37220. Nevertheless, the possible health risks associated with use of these chemicals should be taken in to consideration.

Keeping in mind that mosquitoes have developed resistance to insecticides and that plants are resources of active components for prevention of mosquito bite, the present study was carried out to investigate the repellent activity of the Indian medicinal plant, *Syzygium aromaticum* against the mosquito vector species of malaria *Anopheles stephensi* Liston, which may provide a new source of repellent to protect humans from the mosquito bites. *Syzygium aromaticum* species is a medicinal plant commonly used for antipyretic, anti-inflammatory, antihelmintic, analgesic and it's also useful in abdominal disorders intestinal worms, fevers [15].

## MATERIALS AND METHODS

### Extracts of *Syzygium aromaticum* clove

*Syzygium aromaticum* cloves were procured from the local market and dried in shade and powdered manually. The cloves powder (2.5g) was subjected to extract in a soxhlet apparatus with acetone, hexane and ethyl acetate subsequently in ratio (w/v) of 95% of each at room temperature. The extract was made solvent free and the final residue of each extracts of *Syzygium aromaticum* obtained, lyophilized, and then kept at -20 $^0$C until testing for adult repellent activity. In preparing test concentrations, extracts were volumetrically diluted in absolute acetone and Tween 80 at an appropriate test concentration.

### Mosquito Strains

*An. stephensi* was used in this study. Mosquitoes were reared in a laboratory maintained at temperatures and relative humidity with a photoperiod of hours (Light: Dark) with 90 minutes down and dusk simulation periods. Adult's mosquitoes were provided with 10% sucrose. The 5–8 days old females used for investigation of repellent activity.

### Mosquito Cages

One hundred 5–8 days old-sugar-fed female mosquitoes were introduced in to the cloth cages. These mosquitoes were starved for about 15 hours prior to their introduction in to the cages. A minimum of three replicates were prepared for a given species of mosquito. Needed standardization was made for experiments as to the determination of the suitable age of the mosquito for experiments and method of recording the data [16].

### Preparation of the Repellent and Control Replicates

500 mL of 10% sugar solution was prepared in water. Sufficient quantity of bleached cotton was taken to be stacked in to a 460 mL Styrofoam glass. 460 mL of the above sugar solution was poured into the glass and the cotton was soaked. The cotton at the top was stretched out side in to circular foam. Remaining 40 mL was used to prepare repellent formulations. To 40 mL of the sugar solution required quantities of each extract concentrate were mixed to arrive at the desired concentrations, namely, 2.5%, 5%, and 10% and was poured evenly on the sugar soaked cotton in the above Styrofoam glass. Similarly DEET 2.5% in 10% sugar soaked cotton was prepared for use as positive and only 10% sugar soaked cotton was used as negative controls, respectively. For clove of *Syzygium aromaticum* acetone, hexane and ethyl acetate extracts, known quantity of residue extracts were redissolved in acetone to make a 10% (w/v) stock solution. Various test concentrations ranging between 2.5%, 5%, and 10% were prepared in double distilled water using freshly made stock solution. Controls were supplemented with the equal amount acetone required for the experiment without extracts. Tween-80 was used as an emulsifier at 0.05% concentration in the final test solution.

### Repellency test

Experimental cages with the mosquitoes were placed in this room. In these cages, the Styrofoam glasses with cotton soaked with three different concentrations of acetone, hexane and ethyl acetate extracts of *Syzygium aromaticum* namely 2.5%, 5%, and 10% sugar solution, DEET 2.5% (positive control) in 10% sugar solution and 10% sugar solution (negative control) were placed in four different corners and one in the centre of the cage. Five-minute landing counts were made at 0, 1, 2, 4, 5, and 6 hours. The cups were removed from the cage after the five minute observation at each interval of time. The cup was covered to avoid evaporation of the insecticide formulation and was placed in the refrigerator. For subsequent exposure the position of the cups were inter changed to different corners (16)

### Data analysis

Observation is made in at least three replicates for the given species of the mosquito that landed and attempted to feed were recorded. No mosquito landing occurred in the initial 3 minutes the cups interchanged into the different corners. Observations were made at 30 minutes intervals. If more than 1 mosquito landing was recorded during an observation, the test of repellency was terminated and the

period of repellent protection calculated as the time between the extract application and multiple mosquito landing. The landing rates of the mosquitoes on different concentration of the formulations of acetone, hexane and ethyl acetate extracts of clove of *Syzygium aromaticum* (2.5, 5, and 10 % (DEET 2.5% and sugar (10%) were recorded. Data was reported as mean of the observations for each of the formulations. Results were expressed as average landing counts per exposure interval and as repellency compared to control means using as follows [17]:

Percent protection = [(C-T)/C] x 100

Where C: the mean number of landing on negative control (10% sugar solution); T: landing on the repellents (DEET and clove of *Syzygium aromaticum*).

## RESULTS

The consolidated data of the repellency observed in *An. stephensi* is given and it is evident that the overall repellency rates varied between 66.71–94.78% for different repellents, concentrations of repellents and species. The hexane and ethyl acetate extract of *Syzygium aromaticum* was the candidate for subsequent field investigations repellent activity. Total number of mosquitoes collected from the control is demonstrated in Table 1. Total numbers of 100 adult mosquitoes were reared in the laboratory. Table 1 summarizes the results of three extracts (Hexane, Ethyl acetate and Acetone) of *Syzygium aromaticum* laboratory testing (Figure 1). These results showed a highly significant difference between the mean landings counting on the treated and control in Table 2 and Figure 2. The hexane and ethyl acetate extract of clove showed strong repellent activity against *Anopheles stephensi*. Percent protection obtained against *Anopheles stephensi* (94% in 6 hours) was at the 10% concentration. Against DEET-2.5% An. *stephensi* has shown 100% repellency in 6 hours. However, testing hexane and ethyl acetate extract of *Syzygium aromaticum* need to be carried out in the field.

**Table 1:** Percent repellency of mosquitoes in response to *Syzygium aromaticum* extracts.

| Solvent extract | Doses (%) | Repellency in hours | | | | |
|---|---|---|---|---|---|---|
| | | 0 hour | 1 hour | 2 hours | 4 hours | 6 hours |
| Hexane | Tre-2.5 | 100 | 97.52 | 84.20 | 78.81 | 81.55 |
| | Tre-5 | 100 | 92.49 | 81.59 | 81.81 | 77.77 |
| | Tre-10 | 100 | 92.49 | 89.49 | 84.90 | 77.77 |
| | Deet- 2.5 | 100 | 97.52 | 100 | 94.00 | 92.66 |
| Ethyl acetate | Tre-2.5 | 100 | 100 | 90.71 | 88.41 | 86.88 |
| | Tre-5 | 100 | 95.80 | 90.92 | 93.02 | 92.10 |
| | Tre-10 | 100 | 95.80 | 93.17 | 95.39 | 94.78 |
| | Deet-2.5 | 100 | 95.80 | 93.17 | 96.69 | 92.10 |
| Acetone | Tre-2.5 | 86.23 | 87.50 | 72.71 | 66.71 | 66.71 |
| | Tre-5 | 95.58 | 91.75 | 90.97 | 86.71 | 76.28 |
| | Tre-10 | 100 | 95.87 | 95.49 | 90.57 | 81.00 |
| | Deet-2.5 | 100 | 100 | 95.49 | 95.28 | 100 |

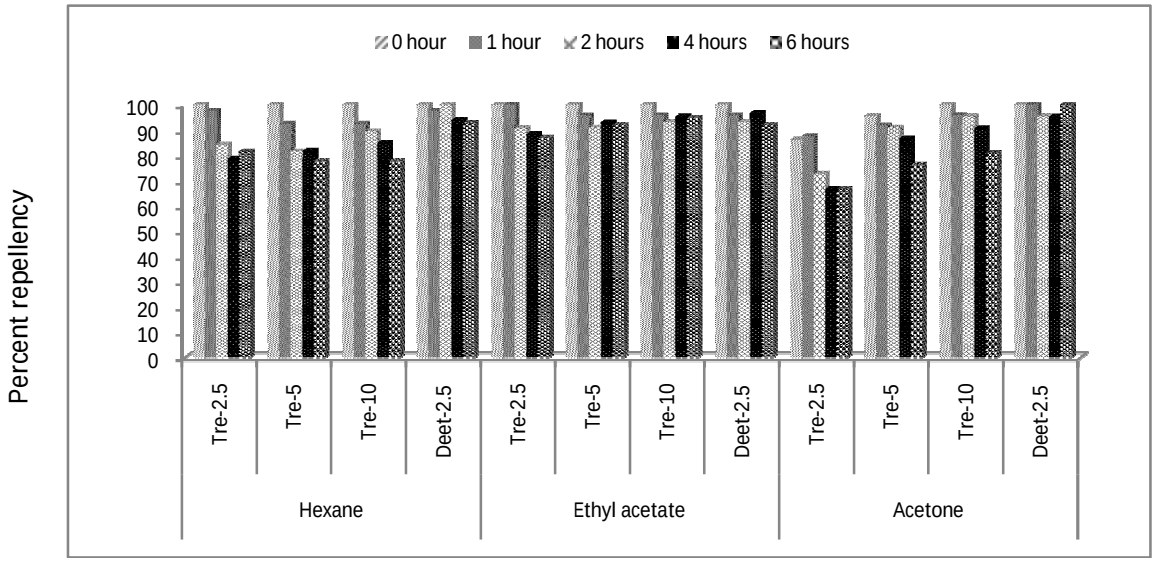


**Fig 1:** Repellent activity of Syzygium aromaticum extracts against *Anopheles stephensi.*

 

**Table 2:** Mean landing of mosquitoes in response to the extracts of *Syzygium aromaticum*.

| Solvent extract | Doses (%) | No. of mosquito landing | | | | |
|---|---|---|---|---|---|---|
| | | 0 hour | 1 hour | 2 hours | 4 hours | 6 hours |
| Hexane | Tre-2.5 | 0.00 | 0.33 | 2.00 | 2.33 | 1.66 |
| | Tre-5 | 0.00 | 2.33 | 2.33 | 2.00 | 2.00 |
| | Tre-10 | 0.00 | 1.33 | 1.33 | 1.66 | 2.00 |
| | Deet- 2.5 | 0.00 | 0.00 | 0.00 | 0.66 | 0.66 |
| | Control | 14.00 | 13.33 | 12.66 | 11.00 | 9.00 |
| Ethyl acetate | Tre-2.5 | 0.00 | 0.00 | 1.66 | 1.66 | 1.66 |
| | Tre-5 | 0.00 | 0.66 | 1.33 | 1.00 | 1.00 |
| | Tre-10 | 0.00 | 0.66 | 1.00 | 0.66 | 0.66 |
| | Deet-2.5 | 0.00 | 0.66 | 1.00 | 0.33 | 1.00 |
| | Control | 18.00 | 16.00 | 7.33 | 14.33 | 7.00 |
| Acetone | Tre-2.5 | 1.33 | 1.00 | 2.00 | 2.33 | 2.33 |
| | Tre-5 | 0.33 | 0.66 | 0.66 | 1.00 | 1.66 |
| | Tre-10 | 0.00 | 0.33 | 0.33 | 0.66 | 1.33 |
| | Deet-2.5 | 0.00 | 0.00 | 0.33 | 0.33 | 0.00 |
| | Control | 9.66 | 8.00 | 7.33 | 7.00 | 7.00 |

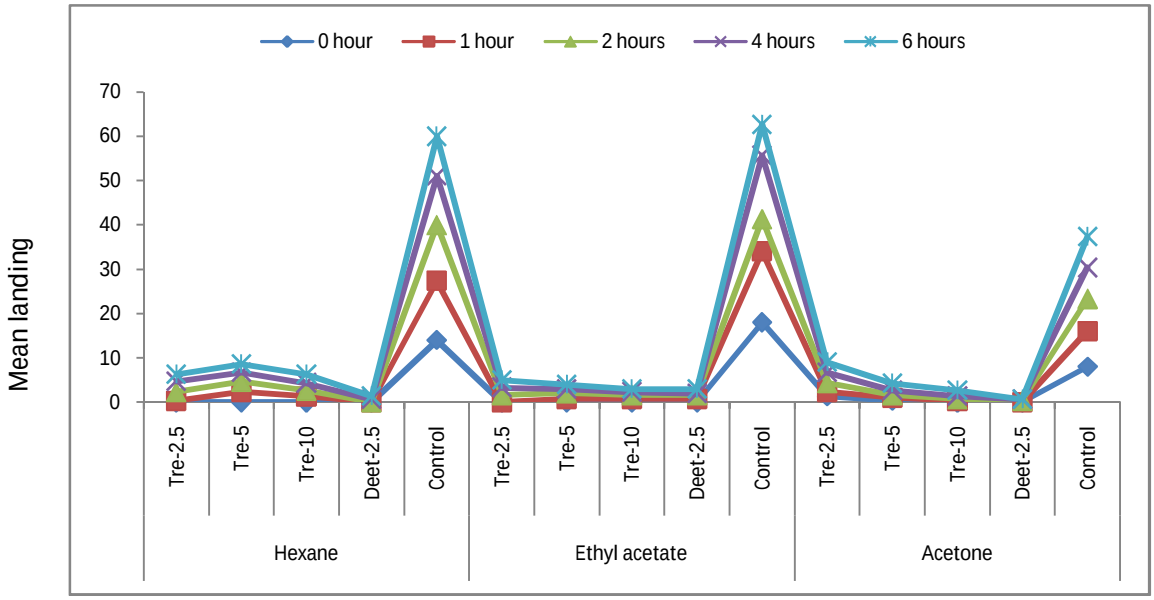


**Fig. 2:** Mean landing of mosquitoes in response to the extracts of *Syzygium aromaticum.*

## DISCUSSION

*Syzygium aromaticum* clove hexane and ethyl acetate extract possessed significant repellent activity against *An. stephensi* in laboratory bioassay, which is similar compared to that reported for used synthetic compounds such as DEET, A13-37220 and CIC-4 [18], [7]. These chemical compounds provide better and longer protection against many biting insects. Repellent protection time in laboratory bioassays however can change depending on the biological characteristics of the mosquito test population. Differences in species and body size, sugar water availability, adult density in test cages, and mosquito age can affect test results [19-21]. Tawatsin et al. [22] demonstrated under laboratory conditions that volatile oils derived from turmeric (*curcuma longa*), citronella grass (*Cymbopogon winterianus*), and hairy basil (*Ocimum americanum*) with the addition of 5% vanillin were effective in repelling both diurnal and nocturnal mosquitoes for up to six hours.

The efficacy of *Syzygium aromaticum* clove extract is comparable to that of currently used commercial repellent product DEET. In recent years, trend for the use of natural repellent products is gaining importance and several botanicals have been tested for the repellent activity against mosquitoes [23-26]. Sharma *et al.* [27] reported the repellent effect of neem oil and showed 37.5% protection against *Cx. quinquefasciatus* with neem oil, whereas in the present study, clove extract of *Syzygium aromaticum* showed upto 98% repellency against *An. stephensi*, but the efficacy declined after one hour. Most of the plant based repellents are shown to repel mosquitoes, but their effect lasts from few minutes to some hours. Their active ingredients tend to be highly volatile, so although they are effective repellents for a

short period after application, they rapidly evaporate leaving the user unprotected [26].The hexane clove extracted *Syzygium aromaticum* may protect against other mosquito vector species. The further studies should be investigated against as many different malaria vector as possible under both laboratory and field conditions. Several methods enhancing the efficacy of repellent, such as purification of the active fraction, increase in persistence and duration of repellency need to be studied.

From the observed data on the repellency against *An. stephensi* malaria vector, it can be concluded that dose of 10% could be used for achieving the desired level of protection against bites of these mosquitoes. However, these results pertain to the effectiveness in cage experiments using only sugar solution as attractant. Thus, further confirmation by testing this repellent on human subjects to evaluate the repellency effect is needed. Our repellent research is being continued to search for the development of new repellents from a natural original that not only offer effective anti-mosquito products but are also bio rational alternatives to synthetic pesticides.

## ACKNOWLEDGEMENT

Authors acknowledge the technical help rendered by Mr. Narender Kumar and G. N Sharma for the completion of the work. We also thank to Dr. T. G. Thomas, National Institute of Communicable Diseases, New Delhi, India for the critical comments in designing the experiment.

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