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A Natural Mosquito Repellent Stick Formulation Using Essential Oils of Plants and Spices

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ABSTRACT

Mosquitoes, as primary vectors of numerous life-threatening diseases, have shown increasing resistance to conventional chemical pesticides, necessitating the development of alternative, eco-friendly control strategies. This study investigates the larvicidal efficacy of a natural essential oil (EOs) blend comprising citronella, clove, eucalyptus, garlic, and neem oils against Aedes aegypti larvae. The essential oil mixture was assessed through bioassays using treatment concentrations of 25, 50, 75, and 100 parts per million (ppm), with 20 Aedes aegypti larvae introduced into each test solution. Mortality rates were recorded after 24 hours, and the lethal concentration (LC_{50}) values were calculated using probit analysis. Results indicated that the EOs mixture exhibited significant larvicidal activity, with the most effective concentration being 50 ppm, corresponding to an LC_{50} value of 53.41. This study not only underscores the potential of essential oil-based larvicides as effective, non-toxic, and environmentally sustainable alternatives but also demonstrates their practical application in the formulation of mosquito repellent sticks. These findings contribute to the growing body of research promoting natural vector control methods that are both economical and ozone-friendly.

Keywords: *Aedes aegypti larvae, Essential oils, citronella, clove, eucalyptus, garlic, neem oils, LC_{50}.*

Introduction

Mosquitoes are the most important arthropod for medical study because they are carriers of several life-threatening diseases like encephalitis, filaria, chikungunya fever, dengue fever, malaria, yellow fever, and West Nile virus infection [1]. In newborns, these infections can lead to serious complications such as encephalitis, high fever, joint pain, and microcephaly. Nearly all tropical and subtropical nations, as well as many other regions of the world, have high rates of mosquito-borne illnesses. Even though there are many mosquitoes that spread disease, only three are deemed dangerous: *Anopheles gambiae*, which spreads lethal malarial parasites; *Culex quinquefasciatus*, which hosts and spreads the West Nile Virus; and *Aedes aegypti*, which spreads the dengue virus. Essential oils derived from a range of plants have been extensively studied to evaluate their mosquito-repelling properties in an effort to address this issue [2]. Garlic oil - *Allium sativum* or garlic has been utilized in traditional medicine for ages in many countries due to its potent fragrance and unique flavour. Repellents need to be persistent and have little volatility on the surface of their host to be effective [3]. Despite their small size, mosquitoes are a serious threat to human health and welfare. In addition to their unpleasant bites, these insects are carriers of a number of dangerous illnesses that can cause widespread sickness and even death.

As new diseases spread by mosquitoes appear, there are more health hazards. For instance, visitors returning from South America have recently been afflicted by the Oropouche virus, which can cause symptoms ranging from fever and headaches to more serious illnesses like Guillain-Barré syndrome and brain enlargement. There is a need for research on vaccines, eco-friendly pesticides, other vector control strategies, and health care worker training initiatives[4].

Materials and Methods

Collection of Mosquitoes

Aedes aegypti larvae were collected from the stagnant water body were reared in laboratory conditions at 25-30°C and 80-90% relative humidity in a plastic tray containing clean unchlorinated water. Larvae were fed on larval food comprising of powdered yeast and powdered dog biscuit (at a ratio of 1:1).

Collection of Plants and Spices

The freshly collected plant leaves of eucalyptus, neem, citronella was collected from kk Nagar, Chennai. The leaves were washed several times with running tap water followed by rinsing thrice with distilled water. The leaves were then chopped into smaller pieces and add it into the round-bottom flask for steam distillation. Likewise, garlic and clove purchased from local market, washed and air dried. It was chopped into small pieces for the extraction of essential oil using steam distillation.

Extraction of oil

Each plant and spice was individually chopped into small pieces for extracting essential oils. A popular technique for obtaining essential oil is hydro distillation (Figure 1) which captures the volatile compounds, most notably diallyl disulfide in garlic oil that give it its unique scent and possible health advantages. The processed samples were then steam distilled separately to extract their essential oils. This essential oil (EOs) was considered as 100% concentration and stored at 4° C for subsequent bioassay on *Ae. aegypti.*

Figure 1: The above image depicts garlic oil extraction through steam distillation using a Soxhlet extractor

All the essential oils (citronella, clove, eucalyptus, garlic and neem) were extracted in the laboratory.

The most widely used method for producing essential oils on a commercial scale is steam distillation. In this type of distillation, vaporized water is poured across plants or flowers that have been placed on a screen. When the steam condenses, water and essential oil are created. The mixture of H_2O and essential oil is finally separated [5]. Ninety-three percent of essential oils may be extracted by steam distillation, with the remaining seven percent being extracted using alternative methods. The heating temperature should be sufficient to liberate essential oils or aromatic chemicals and disperse or break down plant tissue. The most used industrial technique for separating essential oil from plant matter is this one. All the essential oils (citronella, clove, eucalyptus, garlic and neem) were extracted in the laboratory.

Larvicidal Activity of Essential Oils on Mosquitoes

Primary screening was done using the standard method (WHO, 1996). Examining collected mosquito larvae to determine the species they are, their stages of development, and any other pertinent traits is known as screening. Because certain larvicides which are more efficient at particular developmental stages, screening aids in determining the larvae's growth stage. Twenty individuals of early fourth instar larvae were introduced in 500 ml plastic container containing 250 ml of dechlorinated water and desired concentrations of EOs (Table 1).

Essential oil mixture: The following oil concentrations were used to create the stock essential oil mix.

Table 1: Stock preparation of essential oil

S. No	Oil	Volume
1	Citronella	2ml
2	Clove	2.5ml
3	Eucalyptus	2ml
4	Garlic	1ml
5	Neem	1ml

Additionally, dimethyl sulfoxide (DMSO) was added to the oil to improve its solubility. From the stocks 25, 50, 75 and 100 ppm were prepared. For each concentration duplicates were maintained with a total of 100 larvae. Distilled water was used to maintain the larval of early 4^{th} instar larvae. EOs compound toxicity was evaluated at four different concentrations, with mortality ranging from 0 to 100%. In addition to monitoring and documenting symptoms, no food was provided to the larvae following treatment. A larva was classified as dead if it exhibited no swimming movements after 24 hours, even when lightly prodded with a glass rod, following the guidelines outlined in the World Health Organisation's technical report series (WHO, 1999). Mortality rates for each concentration were determined by pooling the number of dead larvae across five replicates. The bioassays were conducted under controlled laboratory conditions at a temperature of 25 ± 5 °C and a relative humidity of 60–70%, using laboratory-reared mosquito larvae [6].

Statistical Analysis

Mortality percentages were corrected for natural mortality using Abbott's formula [7]. The efficacy of the tested oils was evaluated by comparing their LC_{50} values, as well as the slopes of the probit regression lines, following the methodologies outlined [8].

Formulation of Mosquito Repellant Stick

The charcoal powder and wood powder were collected from the local shop it was finely powdered in a mixer and then passed through a sieve [9]. The fine powder was mixed with 100 ppm of essential oil made from the stocks. Total 10 gm of powder premix was taken to prepare 5 incense sticks. Water was gradually added to the fine powder until it attains dough like consistency. The prepared dough was rolled by hand in small quantities in plain sticks. The insect repellent sticks were shade-dried for 24 hrs. under room temperature. The developed mosquito repellent stick was tested for flammability, burning efficiency in relation to burning time, and relative effective repellent activity. The stick's flammability test was then assessed to determine its consistent combustibility, and the duration of burning.

Testing Mosquito Repellent Activity of Incense Sticks Using the Mosquito Net Cage Method

The adulticidal effects of the oils were evaluated using a net cage method bioassay protocol. Adult mosquitoes, aged 3–4 days and fed on glucose, were selected using an aspirator (n = 15 per bottle) and gently introduced into the cages, which were then sealed. After 60 minutes, surviving mosquitoes were transferred to paper cups containing 10% sucrose solution. Mortality was assessed after 24 hours, and the adult mortality rates were analyzed to determine the standardized lethal concentrations (sLC_{50}) using probit analysis.

Results and Discussion

Yield of Essential Oils

This study tested for five essential oils extracted from Citronella (*Cymbopogon nardus*), *Eucalyptus sps*., Clove (*Syzygium aromaticum)*, Garlic (*Allium sativum*), Neem (*Azadirachta indica)* against *A. aegypti* larvae. The table shows the yields of the EOs obtained by hydrodistillation of Citronella, Eucalyptus, Clove, Garlic, Neem leaves. The highest yield was 3.2% of oil was obtained from clove (*Syzygium aromaticum),* where the lowest yield obtained was 1% from garlic (Table 2; Figure 2).

Figure 2: Essential oils (citronella, clove, eucalyptus, neem and garlic) extracted by hydrodistillation

Table 2: Yield of essential oil from plants and spices

Plant	Yield % (V/W)
Citronella (*Cymbopogon nardus*)	0.35
Eucalyptus sps.,	2.1
Clove (*Syzygium aromaticum)*	3.2
Garlic (*Allium sativum*)	1
Neem (*Azadirachta indica)*	2

Similar results were observed from the study [10]. In the study, the essential oils (EOs) were extracted from the leaves of *Cymbopogon citratus*, *Cymbopogon winterianus*, *Eucalyptus citriodora*, and *Eucalyptus camaldulensis* using simple hydrodistillation. Among these, *Eucalyptus citriodora* produced the highest oil yield at 2.10%, whereas *Cymbopogon citratus* yielded the lowest at 0.33%.

Larvicidal Activity of Aedes Aegypti

Primary screening was done using the standard method [11]. The mosquito larvicidal activity of essential oil extracted from Citronella (*Cymbopogon nardus*), *Eucalyptus sps*., Clove (*Syzygium aromaticum)*, Garlic (*Allium sativum*), Neem (*Azadirachta indica)* against *Ae. aegypti.* All five essential oils were blended in various proportions, as outlined in the accompanying table 1, and these mixtures were tested against *Ae. aegypti* larvae with a series of concentration 25, 50, 75 and 100 ppm, respectively. In this procedure, a container with 100 ml of a solution that contained distilled water and the essential oil mixture of citronella, clove, eucalyptus, garlic, and neem oil was used. There were four containers for the larvicidal activity of the essential oil mixture of citronella, clove, eucalyptus, garlic and neem were tested against the fourth instar larvae of *Aedes aegypti* at 25-100ppm (Figure 3).

Figure 3: 20 Aedes aegypti larvae in containers containing essential oil at different concentrations (25–100 PPM)

The number of dead *Aedes aegypti* larvae in their fourth instar (mortality %) at 25–100 ppm was noted after 24 hours. Result revealed that the essential oil possesses varying degree of larvicidal activity against the fourth instar larvae of *Aedes aegypti*. A common metric in toxicology for evaluating the acute toxicity is an LC_{50} (Lethal Concentration $_{50}$) value. LC_{50} (Lethal Concentration $_{50}$) value of this investigation was found to be 31.305 (Table 3). Generally, LC_{50} values between 10-100 is considered to be moderately toxic (Figure 4).

Table 3: Larvicidal activity of each essential oil (EO) evaluated in larvae of Aedes aegypti at 24 h

CONCENTRATION (PPM)	LARVAL MORTALITY (%)[a]	LC_{50}
25	30	31.305
50	50	
75	75	
100	99	

a: Three replicates, a total of 20 larvae each; control mortalities ranged between 0.0 and 2.0%.

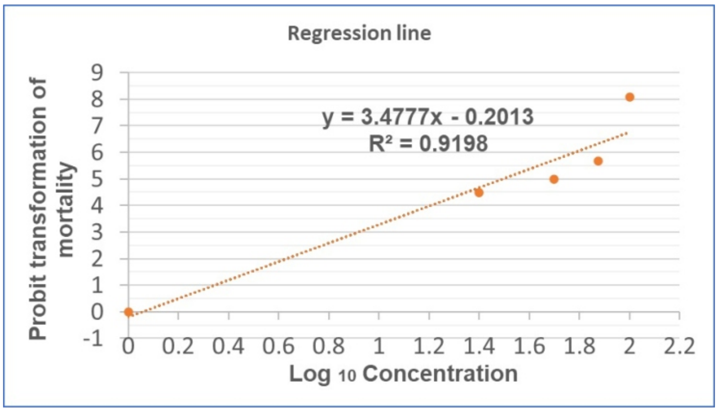

Figure 4: A graph representing the larvicidal activity of the essential oils against Aedes aegypti larvae. (Concentration of 25-100 ppm)

These findings are consistent with previous studies [10]and [12] reported LC_{50} values for *Cymbopogon citratus* essential oil of approximately 120 ppm and 123 ppm, respectively, which closely align with the 120.6 ppm observed in the current study. In contrast, *Cymbopogon winterianus* essential oil demonstrated a lower LC_{50} value of 38.8 ppm. The LC_{50} value of *Eucalyptus citriodora* essential oil was found to be 104.4 ppm in the present study. This is somewhat higher than the value reported and observed an LC_{50} of 70 ppm for the essential oil extracted from *Eucalyptus citriodora* cultivated in Colombia [13]. Consistent with the current findings on larvicidal LC_{50} values, [14] reported an LC_{50} of 216.10 ppm for rosemary essential oil and noted a significantly lower larvicidal potency of peppermint oil, with an LC_{50} exceeding 500 ppm against *Culex pipiens*. Their study indicated that menthol, a saturated monoterpenoid alcohol and the primary constituent of peppermint oil, demonstrated limited larvicidal effectiveness against *Cx.pipiens*.

The larvicidal properties of rosemary essential oil extracted from the aerial parts of *Rosmarinus officinalis* and observed 100% mortality in fourth instar *Cx. Pipiens* larvae at a concentration of 99.34 ppm [15]. The larvicidal activity against *Aedes aegypti, Piper aduncum* essential oil exhibited a lower LC_{50} compared to the other four Piper species considered effective. Previous studies have reported varying LC_{50} values for *P. aduncum* EO against *A. aegypti* larvae, including 46 ppm [16], 50.9 ppm [17], and as high as 289.9 ppm [18]. In the present study, the average LC_{50} value for *P. aduncum* EO against resistant strains (VN and PAMP) and SRL was 25 ppm, which aligns closely with the findings of LC_{50} of 23.73 ppm.[19].

Formulation and Evaluation of Incense Sticks

Incense sticks were prepared according to the procedure given in the materials and methods. The mosquito repellents sticks were prepared by hand roll method and dried at room temperature. Compared to wood, charcoal powder burns more slowly and at a higher temperature, resulting in a longer-lasting and more effective combustion process. Because of this, charcoal powder is especially helpful in places like industrial furnaces and barbecues where a longer, more steady burn is needed. The surface area of wood increases significantly when it is ground into a powder. This facilitates faster and more effective combustion by giving oxygen more surface area to interact with the fuel [20].

Figure 5: Formulation of incense stick with essential oil

Figure 6: Mosquito repellent incense stick made from essential oils

PHYSICAL EVALUATION

The incense sticks were observed to be black in color, and their odour was noted. The texture was assessed by gently pressing the stick. The burning time was found to be 25 ± 5 minutes. The ash weight was 0.156g and the odour was pleasant.

ADULTICIDAL ACTIVITY OF *A. AEGYPTI*

Mosquitoes for this experiment were captured using a large plastic cover and carefully transferred into a mosquito net cage measuring approximately 60 cm × 60 cm. Around 30 mosquitoes were introduced into the cage aligning with their peak biting activity. one fully prepared incense stick was burned in a well-ventilated room, with the mosquito cage placed at the centre. The exposure period lasted for 30 minutes - 1 hour. During this time, the behaviour of the mosquitoes was monitored and recorded. Observations related to mosquito activity (or inactivity) after exposure are presented in Table 4 and illustrated in Figure 7, which shows mosquitoes that displayed no movement following the 30 -minute to one-hour period. Figure 7, provides a visual representation of the mosquito net cage used in the test.

The mosquito showed no movement lying on the floor noted every 15 minutes. Consistent, steady, and complete burning that produces little smoke and may keep mosquitoes away for a longer amount of time are excellent characteristics of an effective mosquito repellent; the number of mosquito deaths is directly correlated with the burning time. It is more preferable to use a mosquito repellent stick with a longer burning time and less weight.

Figure 7: Mosquito net cage for adulticidal activity

Table 4: Adulticidal activity of incense sticks with essential oil (EO)

Concentration (PPM)	Number of mosquitoes with no movement and lying on the floor	Repellency (%)
100	26/30	87

a: Three replicates, a total of 30 larvae each; control mortalities ranged between 0.0 and 2.0%.

similar study was carried out [21]. The mosquito-repellent effectiveness of the formulated incense sticks was assessed and compared with a positive control (Black Dragon). After 4 hours of exposure, formulations F3 and F4 exhibited repellency rates of 89% and 79%, respectively. In contrast, the standard product, Black Dragon, achieved 100% repellency. Among the tested formulations, F3 demonstrated superior repellent activity compared to F4.

Rosemary oil exhibited the highest larvicidal effectiveness among the tested essential oils[22]. In terms of adulticidal and knockdown effects, lavender and peppermint oils proved to be the most potent, each achieving 100% adult mosquito mortality. The median knockdown times (KT_{50}) for lavender and peppermint were 17.43 minutes and 22.81 minutes, respectively. In comparison, rosemary oil resulted in a slightly lower adult mortality rate of 88.89% and a longer KT_{50} of 26.64 minutes. Pulegone was identified as the most potent adulticidal compound among the tested terpenes, with LC_{50} values below 0.1 mg/L against *Aedes aegypti* adults [21]. Significant adulticidal activity, recording an LC_{50} of 14.87 µg/mL against *Culex pipiens* adults, was also reported[23]. Peppermint essential oil has been confirmed to possess effective repellent properties against *A. aegypti* adults[24] .Similarly, lavender and rosemary essential oils have been shown to exhibit strong repellent activity against adult *Culex pipiens pallens* mosquitoes [25].

The adulticidal efficacy of rosemary essential oil against *Culex pipiens* was further supported by findings from its fumigant toxicity to the oil's predominant monoterpene components [26]. However, the study also noted that rosemary oil displayed relatively weak toxicity against mosquito larvae.

The present study clearly showed that the essential oil was more effective at 100 ppm itself. The toxicity of essential oils is significantly influenced by their individual constituents, highlighting the importance of interactions between these components. Such interactions play a crucial role in the development of effective insecticidal formulations.

SUMMARY AND CONCLUSION

Aedes aegypti larvae were collected from the stagnant water body and reared in laboratory conditions at 25-30°C and 80-90% relative humidity in a plastic tray containing clean unchlorinated water. Larvae were fed on larval food comprising of powdered yeast and powdered dog biscuit (at a ratio of 1:1). The freshly collected plant leaves of eucalyptus, neem, citronella was collected and add it into the round-bottom flask for steam distillation. Likewise, garlic and clove purchased from local market, washed and air dried. The yields of the EOs obtained by hydrodistillation of Citronella, Eucalyptus, Clove, Garlic, Neem leaves. The highest yield was 3.2% of oil was obtained from clove (*Syzygium aromaticum),* where the lowest yield obtained was 1% from garlic. All five essential oils were blended in various proportions. These mixtures were tested against *Ae. aegypti* larvae with series of concentration 25, 50, 75 and 100 ppm respectively. The number of dead *Aedes aegypti* larvae in their fourth instar (mortality %) at 25–100 ppm was noted after a 24-hour period. Result revealed that the essential oil possesses varying degree of larvicidal activity against the fourth instar larvae of *Aedes aegypti*. A common metric in toxicology for evaluating the acute toxicity is an LC_{50} (Lethal Concentration $_{50}$) value. LC_{50} (Lethal Concentration $_{50}$) value of this investigation was found to be 31.305.

The mosquito repellents sticks were prepared by hand roll method and dried at room temperature. The incense sticks were observed to be black in color, and their odour was noted. The texture was assessed by gently pressing the stick. The burning time was found to be 25 ± 5 minutes. The ash weight was 0.156g and the odour was pleasant. During this time, the behaviour of the mosquitoes was monitored and recorded 86% repellent activity. This study highlights the potential of five essential oils, as effective larvicides against *Aedes aegypti*. The findings support the use of these oils as eco-friendly alternatives to synthetic insecticides. However, due to their chemical variability, standardised cultivation and formulation practices are essential. Further field trials and safety evaluations are necessary to confirm their effectiveness and safety for non-target organisms.

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