

Original article

Economical novel formulation and evaluation of herbal oils for mosquito and house fly repellent activities

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Abstract

Aromatic plants contain compounds that they use in preventing attack from phytophagous insects with the multiple mechanisms like repellents, feeding deterrents, toxins, and growth regulators etc. Looking at that the present study was carried out with the aim of mosquito and house fly repellent activities with the novel herbal oil formulations. The oils of patchouli, eucalyptus, rosemary, citronella and neem leaves were extracted by hydro distillation method using Clevenger apparatus and various formulations were prepared, viz., tincture, candle and crystal cake. Tincture was evaluated by sprayed in known mosquito larvae and observed for death rate using acetone as control; candle was evaluated on flammability, burning time as well as mosquito and insect repellency test. Furthermore crystal cake formulation was evaluated on appearance, volatility time, stability of fragrance, mosquito and insect repellency test. All the formulations showed remarkable significant dual activities against mosquito and insect population. Based on these preliminary actions, all these formulations were tested in Varthur locality (30 houses and 20 chicken shops) for one month where mosquito and insect populations were more and resulted significant elimination of both the populations. This result may be due to the presence of the active constituents like volatile alcohol, ketone and other constituents in the oils. The result revealed the formulated tincture spray and candle were more effective than crystal cake in relation to killing mosquitoes, insects, stability of fragrance, etc.

Key words: Clevenger apparatus, formulations, herbal oils, physical parameters, mosquito and insect repellency test

1. Introduction

In recent era, vector-borne diseases are spread over the world and chronic infections are transmitted by the infected arthropods, viz., mosquitoes, ticks, bugs, sand flies, black flies and house flies, are serious threat to society for transmission of several life killing diseases. These diseases profoundly restrict socioeconomic status and many of the diseases are located in the tropical and subtropical areas (Bhupen Kalita *et al.*, 2013). Among them, mosquito and fly menaces are a serious global problem and these are increased due to deforestation, industrialized farming and stagnant water (Rani *et al.*, 2013). Malaria, filariasis, Dengue fever, yellow fever, Japanese encephalitis, Ross river virus, Burma forest virus, Murrcey valley encephalitis, chicken guinea, etc., are spread through mosquitoes and reported more than 3 million deaths according to the World Health Organization (WHO) (Ribeiro and Francischetti, 2003; Kaufmann and Briegel, 2004; Harzsch and Hafner, 2006). Thereafter, house flies are very common in Asian countries especially in India. House flies are also carriers of various communicable diseases.

Flies collect pathogens on their legs and mouths when females lay eggs on decomposed feces, garbage and animal corpses, thus populations of flies are increases. Diseases carried by house flies are includes typhoid, cholera and dysentery. Other diseases are like salmonella, anthrax and tuberculosis. They are also transmit the eggs of parasitic worms. The estimated actual burden of cholera is in the vicinity of 3 to 5 million cases and 100 000 to 130 000 deaths per year (Zuckerman *et al.*, 2007). There are several ways to control or destroy the population of mosquitoes and flies by means of chemical treatments like DEET (*N, N*-Diethyl-*meta*-toluamide), DDT (dichlorodiphenyltrichloroethane), can be readily absorbed through the skin, causing many skin poisonings, especially of children. DEET is suspected to be a carcinogen, teratogen and mutagen. They also causes rashes, swelling, eye irritation, and worse problems, though unusual including brain swelling in children, anaphylactic shock, and low blood pressure (Shasany *et al.*, 2000; Phal *et al.*, 2012). Thereafter house flies are controlled by organochlorines, organophosphates, pyrethroids but these chemicals again detrimental to environment and have unwanted side effects an even long term usage of these chemicals developed insects resistance (Thomas and Jespersen, 1994). Even though elimination or eradication of mosquitoes, flies or their larvae, as well as development of economic, less toxic, more effective, human-friendly insect repellants have not received proper focus or attention in the research field but in recent era, the thought is gradually turned towards herbal formulations which are known to be effective

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against a large repertoire of diseases and ailments. Plants are rich source of bioactive organic chemicals which are less toxic, less prone to development of resistance, easily biodegradable and rich storehouse of chemicals of diverse biological activity (Mudrigal *et al.*, 1979). As per the literatures, there are very few natural plants; their formulations are available for insect repellent activities (Rani *et al.*, 2013; Sarvamangala *et al.*, 2014; Bhide *et al.*, 2014; Chavare *et al.*, 2015). Some natural products which effectively repel mosquitoes and flies are reported. Thereafter research evidences are also established on plant based oils, *viz.*, citronella oil, Castor oil, Rosemary oil, lemongrass oil, Cedar oil, peppermint oil, clove oil, geranium oil, oils from verbena, pennyroyal, lavender, pine, cajuput, cinnamon, basil, thyme, allspice, soybean and garlic are have insect repellency activities due to presence of groups of terpenoid active compounds, but they require more frequent reapplication (at least every 2 h.) and higher concentration than chemical ones. Furthermore very few patents are available on mosquito repellents or herbal insect repellents. WHO, World Bank and NGO's are keen to encourage and support preventive and curative research efforts in this field widely. Plant-based repellents are still extensively used traditionally throughout rural communities in the tropics because for many of the poorest communities the only means of protection from mosquito and other insects bites that are available (Moore *et al.*, 2006). Viewed the literature survey, citronella, rosemary, patchouli, eucalyptus and neem leaves were selected in the present study. Citronella (*Cymbopogon nardus*, Family: Poaceae), widely used natural repellents on the market, used at concentrations of 5-10% and the activity due to the presence of constituents like citronellal, citronellol, geraniol, citral, α pinene, and limonene (Curtis *et al.*, 1987). Neem (*Azadirachta indica*, Family: Meliaceae) is a known plant, widely acceptable as a natural alternative to DEET, and it has repellent activity against wide range of arthropods due to presence of azadirachtin, nimbin, nimbidin (Sharma *et al.*, 1993). Rosemary (*Rosmarinus officinalis*, family: Lamiaceae), is well known plant has insect repellency activity due to the oil of rosemary contains bitter principle and volatile oils like borneol, bornyl acetate and other esters (Shooshtari *et al.*, 2013). Eucalyptus (*Eucalyptus globules*, Family: Myrtaceae) oil has much better insect repellent activity because the oil has camphor-like odour and spicy. It possesses a wide spectrum of biological activity including antimicrobial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal, and nematicidal (Batish *et al.*, 2008).

Based on the demand and magnitude of the need for inventing an effective, economic, harmless, safe, non-toxic, mosquito and house fly repellent which can be formulated smoothly and effectively, the present study was aimed with extracted, formulated and evaluated combination of few herbal plant based oils for mosquito and house flies repellent properties.

2. Materials and Methods

2.1 Collection of plant materials

The leaf of the all individual plants were collected from labeled respective plants of the medicinal plant garden of Krupanidhi College of Pharmacy, Bangalore which was earlier authenticated by Dr. Shivananda TN, Principal Scientist, Indian Institute of

Horticultural Research, Hesaraghatta, Bangalore-80. The voucher specimens of all the leaves were preserved in the Pharmacognosy lab of Krupanidhi College of Pharmacy, Bangalore with the respective numbers (KCP/2016-17/01-Citronella; KCP/2016-17/02-Patchouli; KCP/2016-17/03-Neem; KCP/2016-17/04-Eucalyptus and KCP/2016-17/05-Rosemary).

2.2 Extraction of oil

All the plant leaf materials were cleaned with distilled water and size reduced with sharp knife and freshly extracted with cleverger apparatus by hydro distillation method with the temperature controlled heating mantle of 40°C-45°C. Each of the specified plant leaves was weighed about 500 g and extracted for 15 h. Finally the yield was estimated % v/w for respective plants after removed of water by anhydrous sodium chloride salt by kept overnight.

2.3 Collection of mosquitoes and house flies

Various mosquitoes and house flies were collected from garbage area of Varthur village and Gunjur Palya, Bangalore by sweep net method and male and female insects were authenticated by entomology department, Indian Institute of Horticultural Research, Hesaraghatta, Bangalore.

2.4 Selection of experimental zones

There are 10 different village areas were selected in Bangalore where garbages are more and prone to mosquitoes and house flies population. In each area, 10 houses and 10 chicken shops were selected and applied all the formulations (Figure 1).

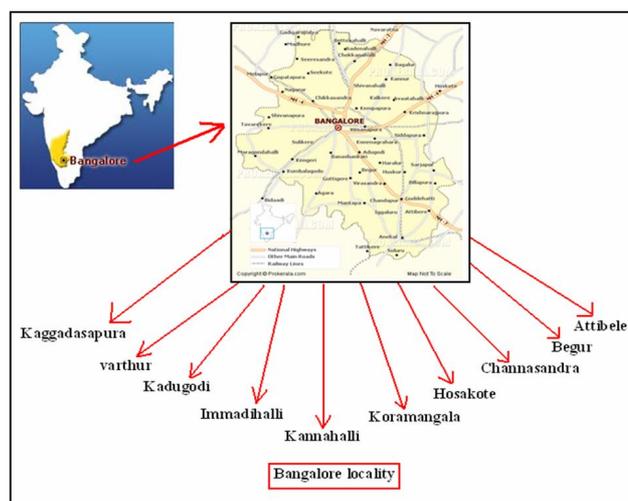


Figure 1: Selected experimental areas

2.5 Preparation of various formulations

2.5.1 Cake formulation

Camphor was accurately weighed and triturated in Grinder and kept aside into a mortar. Measured quantity of extracted Patchouli oil, Neem oil, Eucalyptus oil, Rosemary oil and Citronella oil was added in the empty beaker and mixed to each other with the help of stirrer. Mixed oil was added in to the mortar (containing grinded camphor) and triturated by pastel. Compression of the mixture was done using mild pressure by both hands to form rectangular shape.

2.5.2 Spray formulation

All the extracted oils are mixed in 100 ml of ethanol and poured in spray bottle. The content of oils in formulation was 4 drops of patchouli oil (0.2 ml), 6 drops of neem oil (0.3 ml) and 12 drops of remaining oils of citronella, eucalyptus, rosemary (0.6 ml).

2.5.3 Candle formulation

Paraffin wax was used as a base for candle preparation. The paraffin wax was melted and during liquid condition 2 ml of each mentioned oils were mixed (40 drops) except patchouli oil 0.8 ml (16 drops) and was poured into previously made moulds by papaya straw. Papaya straws are cut into uniform size of 3 inches. At one end the match stick was tied with thread and close with mud. The thread was placed center of the straw and was held until became harder of the formulation containing liquid paraffin. Finally with sharp knife slowly all the straw were cutted out by side and removed the full outer part. Likewise 20 candles were prepared for the present investigation.

2.6 Evaluation tests

2.6.1 Mortality Rate (Das *et al.*, 2003)

The toxicity of the oil was tested against the mosquitoes using well closed 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.

2.6.2 Insect repellent activity

Aerosol bioassay (LT_{50}): Aerosol bioassay was performed according to Umerie *et al.* (1998). The insects were introduced into Peet Grundy Chamber (1 m³). Aerosol sample was sprayed inside the cage as aerosol repellent. Adult mortality was recorded at 5 min interval up to 30 min. A reference control (Allethrin 3 %) was used for comparison. A set of control was maintained in which vapor of deodorized kerosene (DOK) was used. The lethal time (LT_{50}) was recorded from the average of three replicates. LT_{50} were calculated from percentage mortality data using profit analysis (Finney, 1971). At the end of exposure period, the mosquitoes were transferred back to the holding tube and kept 24 h for recovery period. A pad of cotton soaked with 10 per cent glucose solution was placed on the mesh screen. Mortality of insects was determined at the end of 24 h recovery period. Percent mortality was corrected by using of Abbott's formula (Abbott, 1987).

$$\% \text{ Mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

2.6.3 Flammable test

For investigating mosquito and other house fly repellent activities the prepared candle was checked for its flammability, burning efficiency with respect to burning time and eventually its effective repellent activity. Flammability test of candles were conducted to check its consistent combustibility in laboratory condition. Based on the efficiency, further the time taken to burn the candle and its causal effect such as irritation, coughing were observed and recorded in selected mosquito prone areas in the evening and night period such as chicken shops and houses in villages.

2.6.4 Stability test

The essential oils were stored at $26 \pm 2^\circ\text{C}$ in closed vial up to six months and stability of the fraction was determined at 0, and 1, 3 and 6 months time intervals. Whatman no. 1 filter paper (size 10 x 15 cm²) was impregnated with the test fraction at the concentration of 10 mg/cm² during the study. Adulticidal activity was evaluated at $26 \pm 20^\circ\text{C}$ and 60-80% relative humidity.

2.6.5 Public survey

Public volunteer survey carried on 10 houses and 10 chicken shops in selected locality of Bangalore to study safety and efficacy of the formulations. The formulation was distributed to all volunteers and feedbacks were collected. The study was conducted for 1 month. The data was collected and studied statistically.

2.7 Statistical analysis

The values were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) Duncan (Duncan, 1957). The lethal time (LT_{50}) was calculated by profit analysis. A p value of <0.05 was considered as statistically significant. The corrected percent mortality was corrected by using of Abbott's formula.

3. Results and Discussion

3.1 Yield of the oils

The yields of the extracted oils are estimated and the results were tabulated in the Figure 2. The higher yield was calculated 8.21 % v/w for citronella oil, followed by 6.22% v/w for rosemary oil (Figure 2).

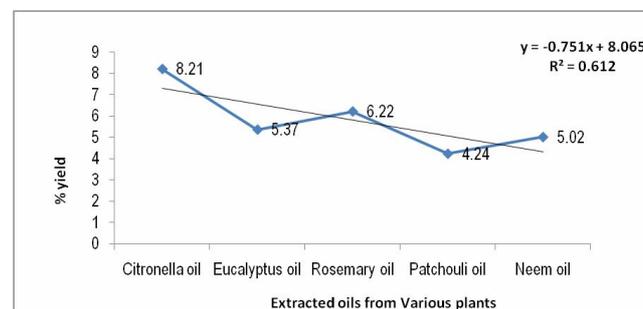


Figure 2: Percentage yield of the oil

3.2 Mortality rate

Control mortality: 7.69 % (2/26)

The mortality rate was depicted in Table 1. Control mortality rate was found to be 7.69%. If mortality exceeds 20% in the control batch, the results of the entire test should be rejected. If mortality in the control is between 5% and 20%, the results with the treated samples should be corrected using Abbott's formula. During investigation, the mortality rate was increased with the time period of contact time of sample at 60 min it shows 79.17 % mortality rate (Figure 3). Same procedure was follows for formulated spray and candles. The result revealed maximum observed mortality was 96.15 % and 92.30 % for spray and candles respectively that are higher than crystal cake formulation (Figure 4). The increase mortality was due to synergistic activity of all the plant that were selected in the present investigation and all the plant are very potent insecticidal.

Table 1: Mortality rate of mosquito repellent of formulated cake

Time (Mins)	Killed (Unit)	Total Unit	Observed Mortality (%)	Corrected Mortality (%)
0	0	26	0	0
10	4	26	15.38	8.33
20	7	26	26.92	20.83
30	10	26	38.46	33.33
40	14	26	53.84	49.99
50	17	26	65.38	62.50
60	21	26	80.77	79.17

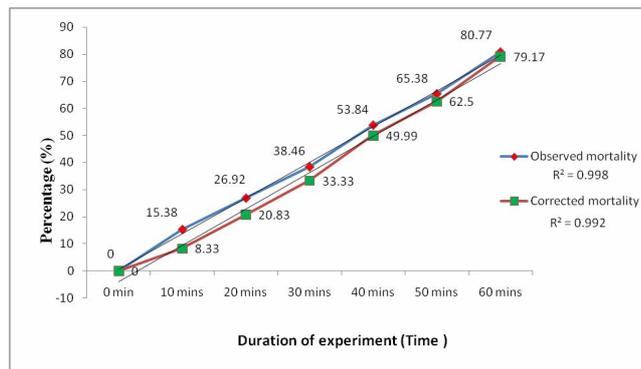


Figure 3: Mortality test for mosquitocidal activity of formulated cake

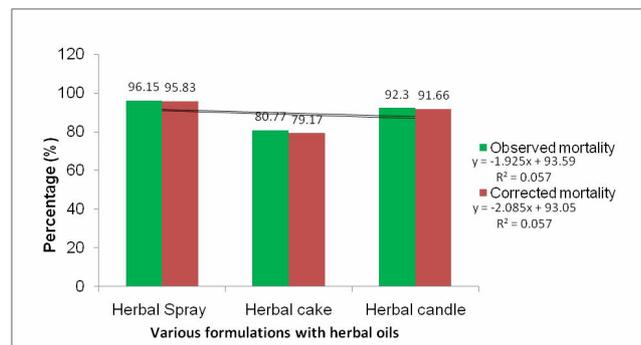


Figure 4: Comparative efficacy of formulated spray, cake and candle based on mortality

3.3 Aerosol assay (LT₅₀)

All essential oils and their mixed formulation in spray were found effective treatments in aerosol bioassay method. These essential oils were tested at four different concentrations, viz., 1.5, 3, 3.5 and 4% /cm³. The results indicated that mixed spray formulation killed 50% populations of adult mosquitoes within 20.2± 0.5 minutes at 4% concentration. LT₅₀ values for house flies ranged from 31.4 ± 0.3 min with 4% concentration (Table 2) when compared with standard allethrin (3%). The LT₅₀ values for all mosquitoes and flies declined exponentially with increasing concentration of essential oils. The results were varied due to the effect of combined oils that showed synergistic effect. Thereafter, typically these oils are liquid at room temperature and get easily transformed from liquid to gaseous state at room or slightly higher temperature without undergoing decomposition. The aromatic characteristics of essential

oils provide various functions for the plants including attracting or repelling insects, protecting themselves from heat or cold and these activities are due to presence of chemical constituents in the oil as well as their utilization. They are constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones and phenols). Essential oils extracted from different families have shown high repellency against arthropod species. Literature has documented that essential oils and extracts from eucalyptus, citronella, rosemary, patchouli and neem have been traditionally used as effective with their potent antimicrobial activities (Kalita *et al.*, 2013; Geetha and Roy, 2014; Akhtar *et al.*, 2014).

Table 2: LD₅₀ study for formulation spray formulation by aerosol bioassay method

Formulation	LT ₅₀ (Mean ± SD) (Lethal time in min) For mosquitoes				LT ₅₀ (Mean ± SD) (Lethal time in min) For house flies			
	1.5	3	3.5	4	1.5	3	3.5	4
Spray	40.1 ± 0.21	33.6 ^c ± 0.02	26.4 ± 0.11	20.2 ^b ± 0.5	48.2 ^{bc} ± 0.02	42.6 ^{ca} ± 0.12	37.4 ^c ± 0.21	31.4 ^b ± 0.3
Allethrin (Standard)	-	4.3 ^a ± 0.11	-	-	-	6.2 ^a ± 0.04	-	-

Mean in each column against each species followed by the different letters are statistically significantly different (p<0.05) by one-way ANOVA with DMRT.

3.4 Flammable test

Each formulated candles were tested in laboratory at uniform normal room condition in confined area where mosquitoes population were more, by lightening and compared with the same size (3 inches) aromatic herbal candle and time noted for the flammability, burning efficiency with respect to burning time and eventually its effective repellent activity. The result revealed more effectiveness and efficacy than the marketed normal candle (Table 3). The higher burning time was observed with more duration of no mosquitoes inside laboratory due to combined used of very potent essential oils such as citronella, neem and patchouli (Maia and Moore, 2011). Research literature earlier stated that citronella candle has more effectiveness for reduction in mosquito bites (Lindsay *et al.*, 1996). Literature also revealed patchouli oil is very dense oil with very strong odour and hence it shows effectiveness against insect in very small quantities (Das, 2016). In formulation, patchouli oil was used very less quantities than other oils.

Table 3: Flammability test for prepared herbal candle

Flammability	Application time	Burning time (min.)	Effective insect repellency
Prepared herbal candle	7.00 pm and 5.00 am	45 ± 0.32	No mosquitoes were surroundings for minimum 2 h.
Marketed repellent aromatic candle	7.00 pm and 5.00 am	32 ± 0.21	Density of mosquitoes less and effective till 1.25 hrs.

3.5 Stability study

All the essential oils separately as well as formulated spray, cake and candles were preserved for stability of their fragrances, color and its efficacy. All the formulations showed no change in physical or any chemical deteriorations even after 6 month study.

3.6 Public survey

Based on the laboratory efficiency, all the formulations were tested

for populated areas for human survey where mosquito and house flies density were more. 10 separate areas were selected where 10 individual houses and 10 chicken shops were selected based on the permission of the people and as per the method experimentation was carried out for a month. As per the survey the result showed tremendous success rate without any side effects. The results were tabulated in Table 4 where assessments revealed best formulation was spray followed by candle.

Table 4: Human survey for efficacy of formulated products (n=10 houses, 10 chicken shops)

Area	Spray DM	TS	Density after using	SE	Candle DM	TL	Density after using	SE	Remarks
Kaggadasapura	70 ± 0.02 %	6.00 pm and 4.30 am	10 ± 0.02%	None	70 ± 0.02 %	8.00 pm	14 ± 0.03%	None	Excellent
Varthur	85 ± 0.1%	6.00 pm and 4.30 am	18 ± 0.02%	None	85 ± 0.1%	7.00 pm	19 ± 0.12%	None	Excellent
Kadugodi	75 ± 0.2%	6.00 pm and 4.30 am	16 ± 0.02%	None	75 ± 0.2%	8.00 pm	17 ± 0.11%	None	Very good
Immadihalli	70 ± 0.02%	6.00 pm and 4.30 am	14 ± 0.03 %	None	70 ± 0.02%	8.00 pm	17 ± 0.11 %	Yes	Nausea reported in one house, Good
Koramangala	78 ± 0.12%	6 pm and 4.30 am	10 ± 0.11%	None	78 ± 0.12%	7.30 pm	14 ± 0.02%	None	Excellent
Kannahalli	80 ± 0.01%	6.00 pm and 4.30 am	12 ± 0.01%	None	80 ± 0.01%	8.00 pm	14 ± 0.11%	None	Excellent
Hoskote	80 ± 0.01%	6.00 pm and 4.30 am	20 ± 0.11%	None	80 ± 0.01%	8.00 pm	22 ± 0.10%	Yes	Skin itching for 2 members in one house, Good
Channasandra	70 ± 0.11%	6.00 pm and 4.30 am	23 ± 0.02%	None	70 ± 0.11%	7.00 pm	24 ± 0.01%	None	Excellent
Begur	70 ± 0.01%	6.00 pm and 4.30 am	20 ± 0.11%	None	70 ± 0.01%	7.00 pm	22 ± 0.10%	None	Very good
Attibele	75 ± 0.02%	6.00 pm and 4.30 am	20 ± 0.10%	None	75 ± 0.02%	8.00 pm	20 ± 0.02%	None	Excellent

DM = Density of mosquitoes before; TS= Time of spray; SE= Side effects; TL=Time of lightning. Density of insects was measured by **house index** method.

4. Conclusion

Novel economical insect repellent formulations were prepared with mixed herbal oils which showed more efficiency without any side effect. The best formulation was herbal spray followed by candle as per the human surveyed. It was also concluded that effectiveness of the product is also depends on the combined application of the herbals rather than single use to get synergistic action. The present investigation leads us to formulate more such products for human use with modern delivery systems to substantial improve in the performance of botanicals for vector and sanitary insect control than synthetic ones, and can easily available in the market with economic price for replacement to synthetic insecticides.

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Conflict of interest

We declare that we have no conflict of interest.

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