

Novel Formulation and Evaluation of Poly Herbal Mosquito Repellent

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Abstract

Aromatic plants produce chemicals that they use to resist phytophagous attacks. Mosquitoes with various pathways, such as repellents, protective eating, metabolites and development controllers etc. Now, present-day conditions result in the burgeoning need for natural mosquito repellents that would have cheap, reliable, non-toxic, environmentally safe, and biodegradable attributes. Driven by the global need to move forward, mosquito repellent candle and mosquito repellent gel formulation based on carbopol 934 was prepared from the *Origanum Majorana*, *Tanacetum Cinerarifolium*, *Juniperus Communis*, Scented Geranium, *Thuja Occidentalis* L., *Tecoma Stans* (L.), *Artemisia Arborescent*, and *Calamintha Acinos* and they are further tested for their size, pH, viscosity, spread ability, extrudability, swelling index and accelerated tests. The prepared candle was tested for flammability and was burnt. Performance with respect to burning time and consequently its efficacy spotting operation. Candle in flammability check was performed to check its consistent laboratory combustibility. Basic therefore, on performance, the time taken to light the candle and its causal symptoms such as pain, coughing has been reported and recorded in identified mosquitoes vulnerable at night and in the field above. Throughout many developed countries, where most residents have little exposure to mosquito net, high-cost mosquito repellent creams, and miscellaneous physical approaches, this formulation of gel may be an efficient, affordable, and easily accessible way to deter mosquito-borne diseases such as malaria, dengue, etc. in the lower parts of society.

Background

Popular insects include mosquitoes, flies, bugs, ticks, butterflies, moths, bees, wasps and so on and are notorious for their unpleasant, itchy sting. The two major classes of insects, that is, insecta and acariens, are primarily interested in vector-borne disease transmission. ^[1] Mosquitoes are typically the best known of the biting insects and potentially the most studied of all arthropods of economic importance. In addition, mosquito-borne diseases such as malaria, chikungunya, yellow fever, Rift Valley fever, dengue fever, and filariasis Japanese encephalitis in many parts of the world produce significant morbidity and mortality in humans and livestock. ^[2] Most insects are known to be major pests attacking humans and domestic animals in order to spread various diseases and some of these diseases may also lead to death. ^[3] Throughout India the most prevalent diseases transmitted by mosquitoes are malaria, chikungunya, dengue, encephalitis and filaria. Any 60 species of *Anopheles* mosquitoes spread the deadly disease, Malaria. According to one World Health Organization (WHO) report in 1990, there were approximately 270 million cases of malaria and approximately one million deaths annually. Lymphatic filariasis is another disease which is spread by insects. It is largely caused by the *Culex pipiens* common house mosquito, and is estimated to be between 100 and 250 million a year. Over the past few decades, the number of individuals at risk from this urban disease has increased. The *Aedes aegypti* is widely spread throughout the globe's tropical, subtropical, and temperate zones, and is one of the largest domestic mosquito species that are both human pests and vectors of disease agents. ^[4]

Classification of Mosquito Repellents-The mosquito repellents currently available can be widely divided into the following categories:(Figure:1)

Physical methods of Mosquito Repellents-The physical methods of repelling mosquitoes act as a protective barrier to mosquito bites. Activities related to mosquito repellent physical methods include emptying the stagnant water into rain gutters, old tyres, buckets, plastic covers, etc. It is highly desirable to change the water in bird baths, fountains, pools, rain barrels etc. on a regular basis at least once a week so that mosquitoes can't find the right place to lay their eggs. It is also recommended that you cover yourself with long sleeved clothing especially during the time of dawn and dusk. One must repair windows and door screens to further prevent mosquitoes from entering the home. This category includes mosquito nets, coils, and other repellents available on the market. Mosquito netting is considered to be a better measure of safety than coils and other repellents particularly during sleep. Such nets serve as a defensive shield to keep humans from being attacked by mosquitoes and other insects. ^[5]

Mechanical methods of Mosquito Repellents-

It was well known that yellow light attracted fewer mosquitoes than white lights and this aspect can be used as a mechanical device for mosquito repellent. Other approaches that fall under the mechanical methods of mosquito repellents include the Electric Mosquito Zapper, Mosquito magnets, etc. An Electric Mosquito Zapper operates by using ultraviolet light to trap mosquitoes and then kills them as they come into contact with their lethal electric charge dose ^[6]

Chemical methods of Mosquito Repellents-

Chemical repellents are the most effective means of personal insect-seeking protection and help deter and control insect-borne disease outbreaks such as measles, Lyme disease, Dengue fever, Bubonic Plague, West Nile fever, etc. ^[7]

This can be further classified into two groups, based on the source of the chemical repellents:

Natural Mosquito Repellents-

Natural resources with proven insecticidal or repellent capacity have played a significant role in interrupting vector-borne disease transmission at both human and community level. Human beings and mosquitoes have existed in close contact from the dawn of human society and the natural resources were used to shield ourselves from mosquito bite and vector borne diseases. These herbal mosquito repellents have been developed and promoted since ancient times, and have led to the discovery of personal protection methods ^[8]

Synthetic Mosquito Repellents-

While plant-derived compounds have long been used as repellents for mosquitoes, they still suffer from certain disadvantages. In contrast with the synthetic repellents, plant related mosquito repellents have

been tested fairly poorly. In comparison, the potency of these herbal repellents appears to last for shorter duration and to fade away easily. Thus, these compounds have short term protection and need regular re-application. Some drawbacks include heavy odor, skin irritation and possible health risks because they were not commonly tested for toxicity. Most significantly, the cost of these repellents is often prohibitive. Extensive research began to find a synthetic replacement for natural mosquito repellents due to the limitations associated with the use of natural plant resources as mosquito repellents, such as limited repellence period and high cost.^[9]

There are many other natural and synthetic substances that have the ability to repel insects, but in the EU and the US, only four primary substances—DEET, Icaridin, Citrioldiol, and IR3535—are recognised for use as insect repellents. Each of them works in a somewhat different way and has somewhat different qualities, but they all produce an odour that repels insects, which is how they all operate. Some important common compounds in insect repellent are mention in figure no.2

THE OBJECTIVE OF THE STUDY- Seeing that most of the mosquito repellent products and devices available on the market have reported adverse human effects, the aim of this analysis is to develop effective mosquito repellent products based on plants.

Methods

Plant selection was based on the study of the various literature and its availability as mosquito repellents with essential oils, experimental data, and folkloric application.

Origanum Majorana, Tanacetum Cinerarifolium, Juniperus Communis, Scented Geranium, Thuja Occidentalis L., Tecoma Stans (L.), Artenmisia Arborescent, And Calamintha Acinos were Purchased from a reliable source.

COLLECTION OF PLANT MATERIAL-

The plant material i.e., *Thuja occidentalis*, *Origanum majorana*, *Juniperus communis* *Tanacetum* collected from natural habitat at udainagar (22.5327° N, 76.2233° E, 370 m altitude India) of Malwa region Madhya Pradesh, India and *cinerarifolium*, *Scented Geranium*, *Tecoma stans*, *Artemisia arborescent*, *Acinos arvensis* (Lam.) were collected from chitrakoot (25.1788° N, 80.8655° E, 137 m altitude India) area in India on October 2017. The use of plants in the present study complied with the institutional guidelines of Dr. A.P.J. Abdul Kalam University, Indore (22.7964° N, 75.9316° E, 557 m altitude, India) and as per the guidelines of Indian Biological Diversity Act 2002 which permits bonafide Indians to access biological resources for scientific research.^[10] The collected plant material is identified morphologically, microscopically and compared with standard Pharmacopeial monograph and the sample of plant was identified & authenticated by Dr. S.N. Dwivedi, Prof. & Head, Department of Botany, Janta PG College, APS University, Rewa (M.P.) INDIA. and assigned with associated voucher specimen numbers. (Table 0.1) They underwent a 45 °C air drying process to maintain a steady weight. The aerial

components were pulverised after drying, labelled and stored in airtight glass at 20 °C until analysis. Then extraction of essential oils was done for the present study.

Table 0.1 Main Group and family of the studied plant species-

Species	Group	Family	Voucher Specimen Number
<i>Origanum Majorana</i> Linn.	Angiosperms	Lamiaceae	J/Bot./OML-236
<i>Tanacetum cinerariifolium</i> Sch. Bip	Angiosperms	Asteraceae	J/Bot./TCF-237
<i>Juniperus communis</i> Linn.	Gymnosperms	Cupressaceae	J/Bot./JCLB-238
<i>Pelargonium graveolens</i> L'Hér.	Angiosperms	Geraniaceae	J/Bot./PGFST-239
<i>Thuja occidentalis</i> L.	Gymnosperms	Cupressaceae	J/Bot.TOL-240
<i>Tecoma stans</i> (L.) Juss. Ex Kunth.	Angiosperms	Bignoniaceae	J/Bot./ TSLF-241
<i>Artemisia arborescens</i> Linn.	Angiosperms	Asteraceae	J/Bot./AAL-242
<i>Acinos arvensis</i> (Lam.)	Angiosperms	Lamiaceae	J/Bot./AAWh-243

Preparation of mosquito repellent hydro gel with carbopol base-

The gel was prepared by combining the constituents in Table 1 taking into consideration the experimental results obtained using arm-in-cage method and the appropriate quantities of the extracts and essential oils. The overall active ingredients in the gel are 12% (V / V per cent). Distilled water was applied to the mix until the final amount was 80.00 ml, and the mechanical stirrer was used to blend all the ingredients for one hour. Eventually, the mixture was poured into plastic containers, and the gel could be set. Formulation of polyherbal gel preparation mention in Figure no 3. ^[11]

Table-1 The Constituents in the Mosquito Repellent Gel.

Constituents	F1	F2	F3
Occidentalis L Essential oil	2ml	2ml	2 ml
Artemisia Arborescent Essential oil	0.6 ml	0.6 ml	0.6 ml
Origanum Majorana Essential oil	2 ml	1 ml	1 ml
Calamintha Acinos Essential oil	1.4 ml	1.4 ml	2 ml
Tecoma Stans Essential oil	2.4 ml	3.4 ml	3 ml
Juniperus Communis Essential oil	1.2 ml	1 ml	1 ml
Scented Geranium Essential oil	2.4 ml	2.6 ml	2.6 ml
Tanacetum cinerarifolium Essential oil	0.8 ml	0.8 ml	0.8 ml
Hexane	1.2 ml	1.2 ml	1.2 ml
Ethanol	1 ml	1 ml	1 ml
Carbapol 934	1 gm	1 gm	1 gm
Propylene glycol	4 ml	4 ml	4 ml
Methylene paraben	0.1 gm	0.1 gm	0.1 gm
Tween 80	3 ml	3 ml	3 ml
Triethanol amine	Few drops	Few drops	Few drops
Distilled water	58.7ml	58.7ml	58.7ml

Preparation of polyherbal mosquito repellent candle-

The candle was composed as the hydrocarbon bases of a mixture of hard paraffin and stearic acid. These constituents are mentioned in table no.2. The recipe for the insect repellent candle is as follows: When burned, the candle was heated by heating (70°C) heavy paraffin and stearic acid. When temperature dropped to 55°C ± 60°C the all-essential oils were added. To get the correct diameter, the wick was plunged repeatedly into liquid wax. The thread was put in the middle of the formed candle. It was inserted into a mold in the shape of a cup, and was then filled with wax. The solidified candle was taken off after cooling. Also 3 different candles have been prepared for the current investigation. Formulation of polyherbal mosquito repellent candle mentioned in figure no.4.

Table-2 The Constituents in the Mosquito Repellent candle.

Constituents	F1	F2	F3
Occidentalis L Essential oil	2ml	2ml	2 ml
Artemisia Arborescent Essential oil	0.6 ml	0.6 ml	0.6 ml
Origanum Majorana Essential oil	2 ml	1 ml	1 ml
Calamintha Acinos Essential oil	1.4 ml	1.4 ml	2 ml
Tecoma Stans Essential oil	2.4 ml	3.4 ml	3 ml
Juniperus Communis Essential oil	1.2 ml	1 ml	1 ml
Scented Geranium Essential oil	2.4 ml	2.6 ml	2.6 ml
Tanacetum cinerarifolium Essential oil	0.8 ml	0.8 ml	0.8 ml
Stearic acid	15gm	15gm	15gm
Hard paraffin wax	QS	QS	QS

EVALUATION OF THE FORMULATED GEL –

1) Appearance: The prepared gels were inspected visually for clarity, colour and presence of any impurities.

2) pH: About 5 ± 0.01 gm of the gel was added to 45 ml of distilled water and stirred to uniform suspension. The pH of the solution was determined by using digital pH meter.

3) Viscosity -Viscosity of the gel was determined by using Brookfield viscometer. Viscosity was measured for the fixed time 60 second for 0.5 rpm. Viscosity determinations were performed at room temperature.

4) Accelerated Stability test

For the span of 90 days, the optimized formulation was subjected to an accelerated stability test ($40 \pm 2^\circ \text{C}$ temperature; $75\% \pm 5\%$, relative humidity). The prepared gel formulation was placed in a PVC container and sealed with a black foil. as assessment of physical appearance, pH, viscosity, spreadability and extrudability.

5) Public survey- Community volunteer survey carried out to test the health and effectiveness of formulations at 10 houses and 10 chicken shops in selected Indore locality. The recipe was circulated to all participants and collected feedbacks. The analysis took 1 month to finish. The results were obtained and systematically analysed.

6) Appearance - The appearance of the cream was judged by its colour, texture, roughness and its odour.

7) Spreadability - The Spreadability was represented in terms of time in seconds by two slides taken to slip off the jelly, placed in between the slides under a certain load. The less time it takes to split the two slides, the greater the quality of the Spread. Two sets of standard sized glass slides have been produced. The herbal cream mixture had been placed on one of the diapers. The other slide was placed on top of the solution, and the cream was placed between the weight of the two slides on the upper slides and the cream was thinly squeezed between the two slides to create a thin sheet. Reduced weight, then brushed off the excess substance adhering to the diapers.

8) Patch Test Approximately 1-3gm of test substance was mounted on a piece of cloth or funnel and added to the vulnerable part of the head, e.g., head behind ears. The cosmetic to be checked has been added to 1sq.m. of the skin region. Command patches were added as well. Within 24 hours the repair site is inspected. The procedure was replicated three times, so there was no response. Since no reaction has been observed on third order, the individual can be taken as not hypersensitive. it is mention in figure no.5. ^[12]

9) Irritancy Test-The prepared 0.5 g gel was added to the usual hairless skin at an area of 6 cm² and then wrapped with a semi-occlusive bandage for 1 hr period. After the application date, the bandage was removed, the applied gel was completely scrapped off and any rashes or related signs were visually examined at the location. The research was administered over 7-day duration. The results were expressed with respect to grades. ^[13]

10) Extrudability- A basic approach to this analysis has been adopted. After the gel was placed in the bottle, the formulas were poured into the collapsible tubes. The extrudability of the various mosquito repellent gel formulations was measured by weight in grams needed to extrude a 0.5 cm of mosquito repellent gel ribbon within 10 seconds. ^[14]

11) Washability The washability of formulations was tested by rubbing the gel on the skin and then determining the ease and degree of washing it with purified water and examining the result manually. ^[15]

12) Swelling index -Taking 2 g of gel in a beaker comprising 10 mL of distilled water determined the swelling index of the prepared polyherbal gel. The swelled formulation was withdrawn from the beaker after 1 hour, and placed on a petridish¹⁸. The substance was measured again and the index of swelling was calculated from the formula:

$$\text{Swelling index (Si)} = \frac{W_t - W_o}{W_o} \times 100$$

Where, W_t = weight of swollen at t time; W_o = original weight of gel at zero time.

Mosquito repellent activity-The volunteers' forearm was immediately thoroughly cleaned with soap and dried up absolutely. The left arm acted as the hand kept inside the mosquito cage. The vector frequency landed on the forearm over a 30-second period. If the mosquitoes have arrived > 10 then the analysis has started. The arm was gently removed from the mosquito cage after a time period of 30 seconds. The right

arm smudged with mosquito repellent gel formulation was consequently joined, and the analysis was completed at 30, 60, 120, 240, and 480 minutes analogously. It was estimated the number of mosquitoes that landed and correlated with the positive control (Odomos Naturals Mosquito Repellent Gel). The analysis was carried out in triplicate format. It is mention in figure no.6.

EVALUATION OF POLY HERBAL MOSQUITO REPELLENT CANDLE-

Flammable test- To research mosquito repelling behaviors the prepared candle was tested for flammability and was burnt Efficiency with regard to burning time and consequently its efficacy Spotting operation. Candle inflammability test was performed to Checks its clear laboratory combustibility. Simple Therefore, on performance, the time taken to light the candle and its Causal symptoms such as pain, coughing has been reported and recorded in identified mosquitoes vulnerable at night and in the field above Period like the chicken shops and village houses.^[16]

Result

All the three gel formulations produced (F1-F3) had a white, less or more porous, homogeneous smooth textured appearance with no firm particles or gritty that was observed while interacting between the fingertips. The translucency of the gel formulations is primarily determined by the concentration of carbopol 934. A reduced proportion of carbopol 934 results in an improvement in the consistency of the gel formulations. No skin irritation, edema, rashes, erythema, or any dermatological reaction or specific inflammation, when applying the gel formulations for 7 days. Additionally, a genius washability attribute was observed for all formulations produced (Table 3)

Table 3: Physical evaluation of the formulated gel formulations.

Formulation	Color	Transparency	Smoothness	Washability	Irritability
F1	White	Less Translucent	Smooth	Good	No Irritation
F2	White	Translucent	Smooth	Good	No irritation
F3	White	More Translucent	Smooth	Good	No irritation

The pH of the gel formulations was observed to be within the range of 7.1-7.3 which is below the usual skin pH level. Viscosity is an essential aspect that affects pharmaceutical properties such as spreadability, extrudability, bottle derivative quality, etc. The formulations' viscosity falls within the range 44600-52900 cps. The rheological study indicated that the shear stress increases extensively with an increase in the torque resulting in a reduction in the viscosity of the formulation. The formulations presented the spreadability within the 14.10-16.73 g.cm/sec range which reflected that a small amount of

shear would easily spread the gel formulation. A quantitative analysis of spreadability and viscosity showed that the spreadability decreases dramatically with an increased viscosity of the formulation. The prepared gel formulations displayed impressive extrudability with a large amount of extruded content (+ + to + + +). Despite the formulation's increased viscosity, the extrudability increases alongside and inhibits fast extrusion from the collapsible pipeline. The swelling index of 110-123 per cent was observed. The swelling index indicated the matrix existence of the gel formulation which inhibits the drug's-controlled release. (Table 4)

Table 4 Evaluation parameters of polyherbal gel formulations.

Formulation	pH	Spreadability (g.cm/sec)	Viscosity (cp)	Extrudability	Swelling index (%)
F1	7.4	16.73	52600	++	110
F2	7.3	15.55	49900	++	115
F3	7.2	14.10	43600	+++	123

No major differences in pH, viscosity, spreadability, swelling index, extrudability, and physical appearance were observed when the optimized formulation (F3) was exposed for 90 days at accelerated conditions ($40\pm 2^\circ\text{C}$ and 75 ± 5 percent RH). Changes in pH by 0.1 percent, viscosity by 600 cps, swelling index by 9%, spreadability by 0.79 g.cm/sec were found significantly (table 5) Nevertheless, after research, no improvements in the physical appearance, translucency, and smoothness were observed. Overall, the formulation remained constant for the 3-month cycle and in tropical and subtropical regions is predicted to remain in its original form for a longer time.

Table 5: Accelerated study of optimized gel formulation (F3).

Duration	pH	Spreadability (g.cm/sec)	Viscosity (cp)	Extrudability	Swelling index (%)
0th day	7.1	16.73	44800	+++	123
90th day	7.0	17.56	42000	+++	114

The F3 formula demonstrated the maximum 90.45 percent mosquito repellent activity in the 0th hr, and proceeded to work up to 88.35 percent in the 3rd hr. (Table 6) The other formulations F1 and F2 displayed

less operation 83.22% to 85.66% after 3 hours, while the standard product (Odomos natural's mosquito repellent gel) demonstrated almost 98% efficacy after the 3rd hour lapse.

Table 6: Mosquito screening results of the formulated gel.

Treatment stability	Repellency (%)			
	0hr	1 hr	2 hr	3hr
Positive control	100±1.92	99.68±1.23	97.88±1.86	97.78±1.44
F1	86.27±1.89	84.48±1.26	83.45±1.25	83.22±1.23
F2	88.38±1.49	87.48±1.88	86.17±1.76	85.66±1.45
F3	90.45±1.29	89.88±1.65	88.74±1.92	88.35±1.88

In patch test Within 24 hours the repair site is inspected. The procedure was performed three times, since there was no response. Since no reaction on third application has been detected, the individual can be taken as not hypersensitive. (Table 7)

Table 7 Mosquito repellent gel patch test

Patch test		(Hypersensitivity)		
Formulation	1 st 24 hr	2 nd 24hr	3 rd 24 hr	
F1	NO REACTION	NO REACTION	NO REACTION	
F2	NO REACTION	NO REACTION	NO REACTION	
F3	NO REACTION	NO REACTION	NO REACTION	

Flammable test- Flammable test of poly herbal mosquito repellent candles are mention in table no.08. Each formulated candles were tested in laboratory at uniform normal room condition in confined area where mosquitoes' population were more, by lightning 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 candle (Aura Decor Anti Mosquito & Bug Repellent Jar Candle). Owing to the mixture of very active essential oils such as Origanum Majorana, Tanacetum Cinerarifolium, Juniperus Communis, Scented Geranium, Thuja Occidentalis L., Tecoma Stans (L.), Artemisia Arborescent, And Calamintha Acinos, the higher burning time was found within the laboratory. These all-essential oils are more effectiveness for mosquitoes.

Table-8 Flammable test of poly herbal mosquito repellent candle

Flammability		Application time	Burning time (Min.)	Effective mosquito repellency
Marketed mosquito repellent aromatic candle		7:00pm and 5:00am	60	Lower and efficient mosquito density till 1.25 hrs.
Prepared poly herbal mosquito repellent candle	F1	7:00pm and 5:00am	90	There were no mosquitoes around for a min of 3 hours.
	F2	7:00pm and 5:00am	90	There were no mosquitoes around for a min of 2.30 hours.
	F3	7:00pm and 5:00am	90	There were no mosquitoes around for a min of 2.30 hours.

The F1 formula demonstrated the maximum mosquito's repellency compare to the F2, F3 and marketed polyherbal mosquito repellent candle.

PUBLIC SURVEY OF FINAL PRODUCT-

All the formulations were tested on the basis of the laboratory performance for human sample inhabited areas where mosquitoes and households are Density of flies became higher. 10 Separate areas where 10 were selected Specific houses and 10 shops for chicken were chosen based on the People's permission and as per experimental process It was executed for a month. The findings showed according to the survey Significant success rate, with no side effects. The result was Tabulated in Table 9 where best formulation was revealed was a candle and gel.

Table-9 Human survey for efficacy of formulated products (n=10 houses, 10 chicken shops)

Density of insects was measured by house index method.

Area	Gel DM (%)	TS	Density after using (%)	SE	Candle DM	TS	Density after using (%)	SE	Remark
SouthTukogaj	75 ± 0.06	6.00 pm and 6.30 am	10 ± 0.03	None	75 ± 0.06	8.00 pm	12 ± 0.03	None	Excellent
Vijay nagar	90 ± 0.5	6.00 pm and 6.30 am	15 ± 0.03	None	90 ± 0.5	8.00 pm	15 ± 0.12	None	Very good
Simrol	75± 0.03	6.00 pm and 6.30 am	17 ± 0.04	None	75± 0.03	7.00 pm	16 ± 0.10	None	Excellent
Datoda	80± 0.13	6.00 pm and 6.30 am	12 ± 0.01	None	80± 0.13	7.30 pm	16 ± 0.11	None	good
Nehru nagar	85 ± 0.02	6.00 pm and 6.30 am	10 ± 0.18	None	85 ± 0.02	8.00 pm	12 ± 0.04	None	Very good
Shivaji nagar	84 ± 0.05	6.00 pm and 6.30 am	14 ± 0.04	None	84 ± 0.05	8.00 pm	14 ± 0.10	None	good
Dhar road	70 ± 0.15	6.00 pm and 6.30 am	15± 0.10	None	70 ± 0.15	7.30 pm	20 ± 0.10	None	Excellent
Rau	72 ± 0.01	6.00 pm and 6.30 am	20 ± 0.04	None	72 ± 0.01	8.00 pm	22 ± 0.04	None	Very good
MR 10	70 ± 0.02	6.00 pm and 6.30 am	15± 0.08	None	70 ± 0.02	7.00 pm	22 ± 0.10	None	Very good

LIG	78 ± 0.04	6.00 pm and 6.30 am	22 ± 0.12	None	78 ± 0.04	8.00 pm	18 ± 0.05	None	Excellent
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Discussion

Many studies have demonstrated that plant essential oils are more effective repellents than plant extracts. All essential oils, on the other hand, are quite powerful. This makes them unstable, which adds to their short lifespan. As insect repellents as a result, numerous essentials are required. Oils are not acceptable for use as a solo mosquito repellent. Repellents. This issue is addressed in the current report. Prepare two natural mosquito repellents as part of your research.

compositions using essential oils that are very volatile with the use of herbal extracts. The extracts, essential oils, and two insect repellent formulations caused no skin irritations or rashes on the test participants' arms.

Clove bud essential oil, on the other hand, caused a burning sensation on the test participants' arms. As a result, the small burning sensation felt on the arms of the test participants after applying two mosquito repellent formulations might be attributed to pyrethrum essential oil. Reducing the Clove bud essential oil to the point where no scorching feeling is felt will enhance the two insect repellent formulas even further. Additionally, formulations with lower concentrations of active substances must be produced and tested for effectiveness.

Conclusion

Natural base mosquito repellent was successfully developed in this research study. The drug has been found to be very effective and safe to use. Owing to its beneficial use in the human body, the prepared eight separate essential oil-based mosquito repellent gel and candle formulations have proven to be an effective tool in preventing mosquito-borne diseases. In many developed countries, where most residents have no access to mosquito net, high-cost mosquito repellent creams, and miscellaneous physical methods, this formulation of gel and candle can be an efficient, affordable, and easily accessible way to deter mosquito-borne diseases such as malaria, dengue, etc. in the lower parts of society.

Abbreviations

DM = Density of mosquitoes before; TS= Time of spray; SE= Side effects; TL=Time of lightening=Resolution per minute: PVC =Polyvinyl chloride: °C=Celsius: D.S-Dark skin, F.S –Fair skin, M.S- Medium skin: Wt = weight of swollen at t time; Wo = original weight of gel at zero time.

Declarations

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Studies involving plants: The plant material i.e., *Origanum majorana*, *Tanacetum cinerarifolium*, *Juniperus communis*, *Scented Geranium*, *Thuja occidentalis*, *Tecoma stans*, *Artemisia arborescent* and *Acinos arvensis* (Lam.) were collected from various study sites of Malwa region of Madhya Pradesh(India) and were authenticated by Dr. S.N. Dwivedi, Prof. & Head, Department of Botany, Janta PG College, APS University, Rewa Madhya Pradesh(India). Voucher Specimen No. J/Bot./OML-236;J/Bot./TCF-237;J/Bot./JCLB-238;J/Bot./PGFST-239;J/Bot.TOL-240;J/Bot./ TSLF-241;J/Bot./AAL-242 and J/Bot./AAWh-243

Author contributions: DKG conceptualized and drafted the manuscript and also designed the experiment. AT was a major contributor in editing and revising with help in collection of plant and prepared figures 1–2. RG substantively reviewed the draft with given idea of Development of the both formulations. All authors read and approved the final manuscript.

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Availability of data and materials: All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate: The study including sample collection was conducted according to India's Biological Diversity Act 2002 which permits bonafide Indians to use biological resources for scientific research. ^[10]

Consent for publication: Yes

Competing interests: The authors declare that they have no competing interests.

References

1. Mimms C. A., Playfair J. H. L., Roitt I. M., Wakelin D., R., Williams In: Clinical Microbiology, Europe Limited, 1993, Mosby Year Book.
2. Eldridge B. F., Edman J. D., In: Introduction to medical entomology. Medical Entomology: A Textbook on Public Health and Veterinary Problems Caused by Arthropods, Eds B. F. Eldridge, J. D. Edman (Kluwer, London), 2004 p 1.
3. Olkowski W., Larval Mosquito management, Common Sense pest Specific Quarterly, 2001, 17, 8.
4. Halstead S. B., Dengue Bull. 2000 24, 77.
5. <http://www.mosquito netting.com/mosquito control.html>
6. Enayati A. A., Hemingway J., Garner P., Cochrane Database Syst. Rev2007. 18, 34.

7. Mishra, A. Singh K. N., Sharma V. P., Indian J Malariol, 1995, 32 (3), 99.
8. ICMR Bulletin, Prospects of using herbal products in the control of mosquito vectors, 2003, 33 (1), 1.
9. Peterson C., Coats J., Pesticide Outlook, 2001 12, 154.
10. Venkataraman K. India's Biodiversity Act 2002 and its role in conservation. Tropical Ecology. 2009 Jun 1;50(1):23.
11. Ranasinghe MS, Arambewela L, Samarasinghe S. Development of Herbal Mosquito Repellent Formulations. Int J Pharm Sci Res, 2016; 7(9):3643-3648.
12. Vijayalakshmi A., Tripura and Ravichandran A. V.; Development and Evaluation of Anti- Acne Products from Terminalia arjuna Bark; IJCRGG Vol. 3, No.1, pp 320-327.
13. Bhowmik Debjit, Gopinath Harish, B. Kumar Pragati, Duraive S. I, G Aravind., K. P. Kumar Sampath; Medicinal Uses of Punica granatum and Its Health Benefits; Journal of Pharmacognosy and Phytochemistry; 2013, Vol. 1 No. 5.
14. K.R Vinod, Santhosha D, Anbazhagan. S; Formulation and Evaluation of Piperine Creama New Herbal Dimensional Approach for Vitiligo Patients; Int J Pharm PharmSci, 2011, Vol 3, Suppl 2, 293.
15. Gupta R, Gupta GD. Formulation Development and Evaluation of Anti-inflammatory Potential of Cordia oblique Topical Gel on Animal Model. Pharmacogn J, 2017; 9(6): s93-s98.
16. Das Kuntal, Vasudeva C. Vasudeva and Dang Raman. Economical novel formulation and evaluation of herbal oils for mosquito and house fly repellent activities. Annals of Phytomedicine, 2016: 5(2): 91-96.

Figures

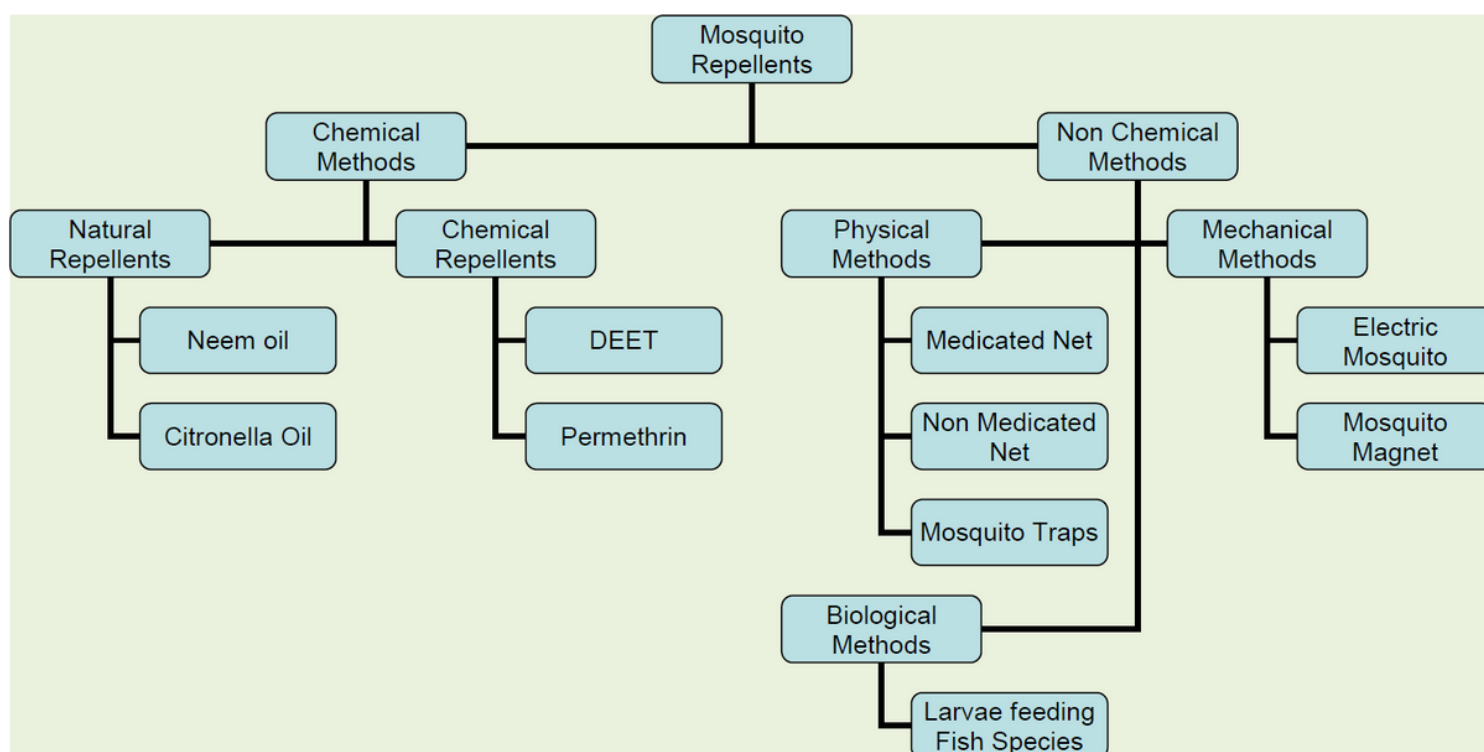


Figure 1

Broad Classification of Mosquito Repellent Methods

THE CHEMISTRY OF INSECT REPELLENTS



WHAT ATTRACTS INSECTS?
1-OCTEN-3-OL

CCCCC/C=C/C(O)C

This chemical is contained in human sweat and breath, and is thought to attract biting insects such as mosquitos. It is often used in combination with CO₂ in mosquito traps. It is also found in mushrooms, and sometimes called 'mushroom alcohol'.

COMMON COMPOUNDS IN INSECT REPELLENTS

DEET
N,N-DIETHYL-M-TOLUAMIDE

CCN(CC)C(=O)c1cccc(C)c1

- Most common active ingredient.
- 2-8 hours protection (20-30% solution).
- 10-30% solution safe for adults. <10% recommended for children up to 12.
- Effective vs. mosquitos, ticks, fleas & other biting insects.

ICARIDIN
A.K.A PICARIDIN

CCOC(=O)N1CCCCC1CCO

- Odourless & doesn't damage plastics.
- 2-8 hours protection (20% solution).
- Not a skin irritant, but can cause mild eye irritation on contact.
- Effective vs. mosquitos, ticks, fleas, biting fleas & chiggers.

CITRIODIOL
P-MENTHANE-3,8-DIOL

CC1(C)C(O)CC(C)CC1O

- From oil of the Lemon Eucalyptus tree.
- ~6 hours protection (10-20% solution).
- No adverse effects - irritation possible if contact with eyes occurs.
- Mosquito, fly & gnat repellent; also a miticide, killing insects and mites.

IR3535
ETHYL 3-[ACETYL(BUTYLAMINO)]PROPANOATE

CCCCN(C)CC(=O)OCC

- No negative effects reported.
- 2-6 hours protection (10-20% solution).
- Not harmful when ingested or inhaled, though can irritate eyes on contact.
- Effective vs. mosquitos, ticks, body lice and biting flies.

HOW DO REPELLENTS WORK?



Recent studies suggest that, after initially being exposed to the repellent DEET, insects can temporarily overcome or adapt to its repellent effect. This could have future implications for how the efficacy of repellents is determined.

A BUILT IN DEFENCE?
1-METHYLPYPERAZINE

CN1CCNCC1

Researchers have found that compounds found naturally on the skin, either as a result of secretions, or surface bacterial activity, are able to render humans 'invisible' to mosquito senses. One of these compounds is 1-methylpiperazine.

Figure 2

Chemistry of insect repellent

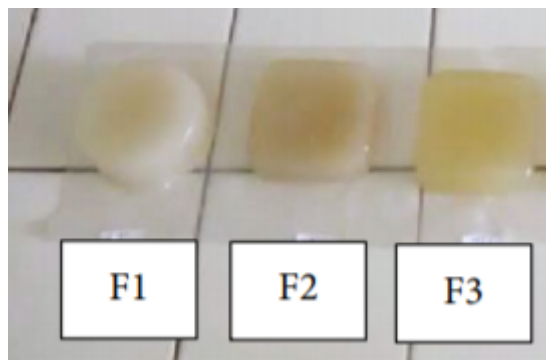


Figure 3

Formulation of polyherbal mosquito repellent gel



Figure 4

Formulation of polyherbal mosquito repellent candle



Figure 5

Patch test

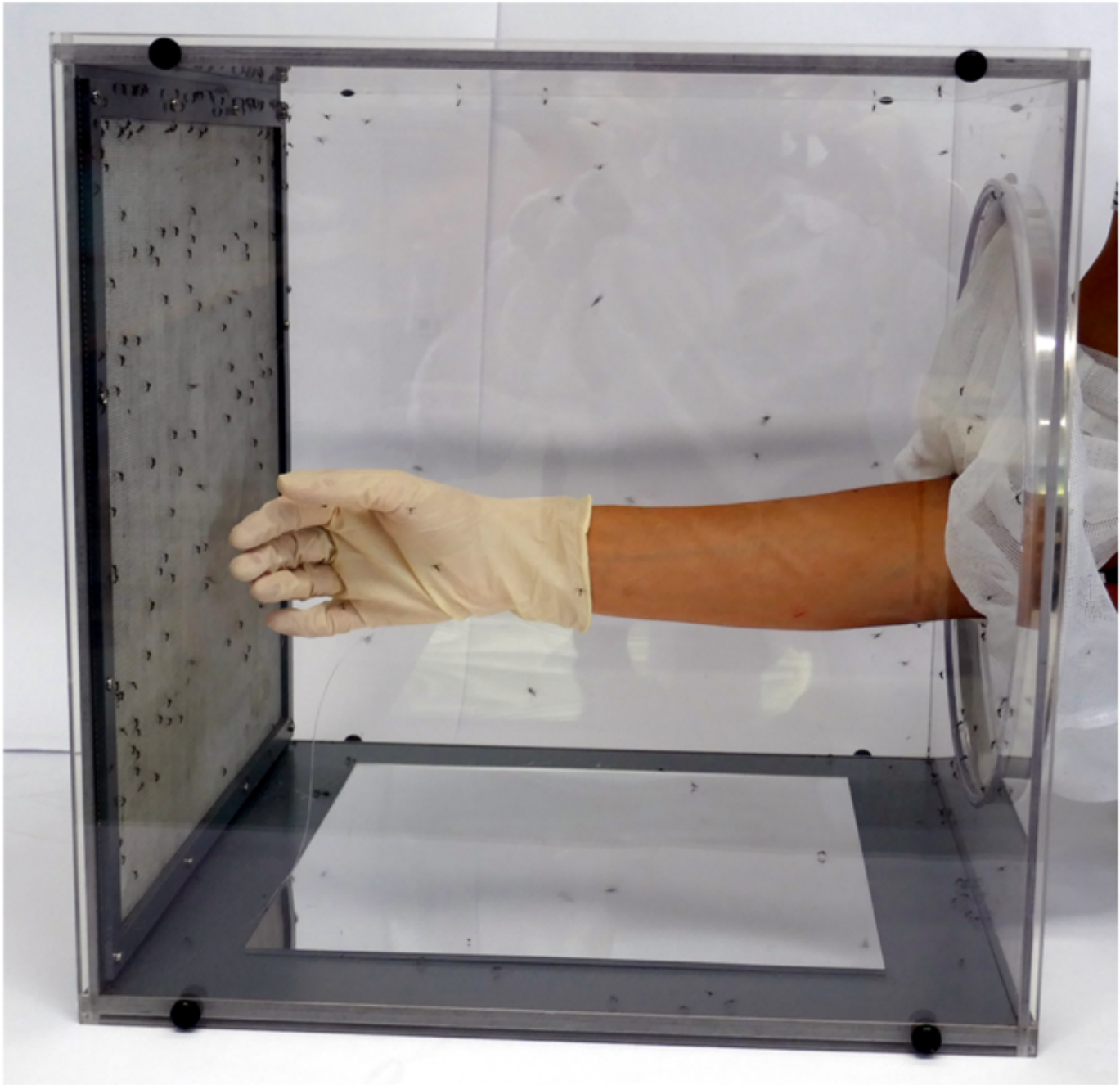


Figure 6

The arm-in-cage (AIC) test for measuring the efficacy of topical mosquito repellents Under laboratory conditions

Supplementary Files

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