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Research Article

Development of Herbal Mosquito Repellent Spray and Candle Formulation

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ABSTRACT

Mosquitoes are found all throughout the world and are important carriers of diseases such as malaria, dengue, and yellow fever. There are numerous Chemical based mosquito repellents on the market, but they are linked to numerous negative consequences and may be hazardous to both people and the environment. The creation of natural repellents substitutes can greatly benefit from and have been Knowledge of traditional repellent plants. Numerous physiologically active substances found in herbal plants and have anti- mosquito properties, because herbal compounds are safe and effective and Nontoxic, their uses are growing in popularity. The research for this study entails choosing appropriate herbal raw material creating the product and assessing how well the spray and candle repel mosquitoes. The safety of the spray and candle is guaranteed by the use of natural herbal substances and essential oils, including Tulsi, clove, eucalyptus globules, Neem, Citronella which are known for their ability to repel mosquitoes.

INTRODUCTION

Mosquitoes are among the most disturbing blood sucking insects afflicting human beings. Several mosquito species belonging to genera Anopheles, Culex and Aedes are vectors for the pathogens of various diseases like Dengue fever, Malaria, Yellow fever, Japanese Encephalitis and several other infections. Malaria which is caused by Plasmodium parasites transmitted through the bites of female Anopheles mosquitoes continues to impart a major disease burden on infants and young children. The Aedes aegypti mosquito which spreads Dengue fever is responsible for more than 100 million infections worldwide every year, leading to thousands of deaths[2].

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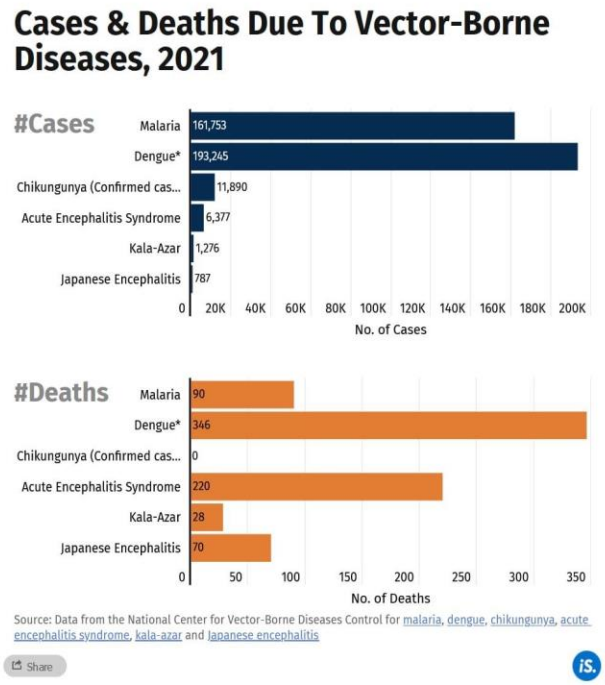


- Mosquitoes alone transmit disease to more than 700 million people and over one million deaths are reported annually across the globe.

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Diptera
Family: Culicidae

- The majority of commercial repellents are made using chemicals such as N, N-diethyl-meta toluamide (DEET), allethrin, N, N-diethyl mendelic acid amide, and dimethyl phthalate.
- However, it has been determined that chemical repellents are not safe for public health due to observed toxic reactions. Utilizing traditional plant knowledge to develop novel natural repellents as an alternative to chemical-based repellents is highly valuable.
- Therefore, mosquito control research is crucial to halt the spread of diseases transmitted by mosquitoes and to protect the environment from the use of chemical pesticides.
- This project focuses on the formulation and evaluation of a herbal "mosquito repellent candel" and "mosquito repellent spray" candels offer a convenient and pleasant method of repelling mosquitoes. By incorporating natural herbal ingredients and essential oils known for their mosquito repellent properties such as citronella,neem,clove,eucalyptus,orange peel we aim to create a formulation that is both safe and non- toxic.

Primary Disease-

1.Aedes aegypti: Transmits Dengue, Yellow Fever, Chikungunya, and Zika virus.

2.Aedes albopictus: Transmits Dengue, Chikungunya, and Zika virus.

3.Anopheles: Transmits Malaria.

4.Culex: Transmits Lymphatic Filariasis, Japanese Encephalitis, and West Nile Virus[3].

- **Lifecycle of Mosquitoes**

The lifecycle of a mosquito consists of four distinct stages:
egg, larva, pupa, and adult

Here's an overview of the lifecycle of a mosquito:

***Stage 1:** Egg (3-5 days)*

1. Female mosquitoes lay eggs in standing water, such as ponds, lakes, or even small containers.
2. Eggs hatch into larvae within 3-5 days.

***Stage 2:** Larva (5-7 days)*

1. Larvae, also known as wrigglers, feed on tiny organisms and organic matter in the water.
2. Larvae molt four times as they grow and develop.

***Stage 3:** Pupa (2-3 days)*

1. When the larva is fully grown, it transforms into a pupa, also known as a tumbler.
2. Pupae rest at the water's surface, breathing air through a siphon.

***Stage 4:** Adult (1-2 weeks)*

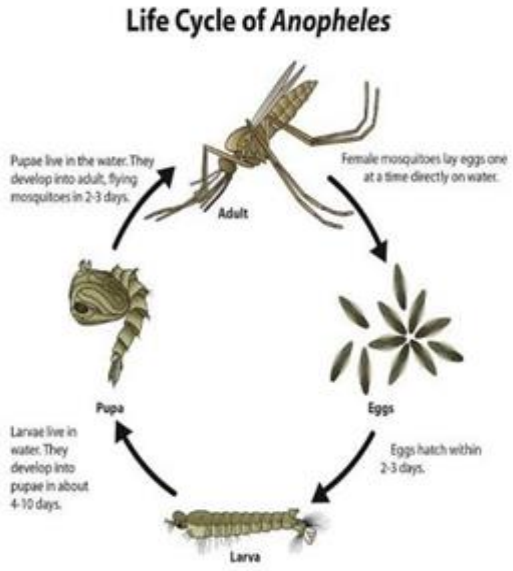
1. Adult mosquitoes emerge from the pupal stage, their wings still soft and folded.
2. Adults feed on nectar and mate, with females seeking a blood meal to produce eggs.
3. Adult mosquitoes can live for several weeks, with some species living up to a few months.

***Female Mosquito's Life Cycle: ***

1. Female mosquitoes can lay multiple batches of eggs throughout their lifetime.
2. After laying eggs, females seek a blood meal to produce more eggs.
3. This cycle repeats until the female mosquito's death.

Male Mosquito's Life Cycle:

1. Male mosquitoes typically live for a shorter period than females.
2. Males focus on mating and do not bite humans or animals for blood.
3. After mating, males usually die soon after.



Prevention and Control of Mosquito-Borne Diseases

- Mosquito Repellents
- Mosquito nets
- Eliminate breeding sites
- Regular cleaning
- Clothing
- Vaccines and medication[4].

Mosquito Repellents:

- Mosquito repellents are substances designed to deter mosquitoes from approaching or landing on surfaces, particularly human skin They are substances applied to skin, clothing or other surfaces.
- They work by making the treated area unattractive or offensive to mosquitoes, thereby reducing the bites and potential transmission of mosquito-borne diseases such as malaria, dengue fever, and Zika virus.

- **Types of Mosquito Repellents**

Chemical Repellent: DEET, Picaridin, Ethyl butyl acetyl
aminopropionate.

Natural Repellents: Oil of lemon eucalyptus, citronella,
other essential oils (Lavender oil, Rosemary oil, neem oil)

Wearable Repellents: Repellent wristbands, Clip-On
devices.

Spatial Repellent: Candles, Coils, Dhoops, Sticks, Electric
diffusers and plug-Ins.

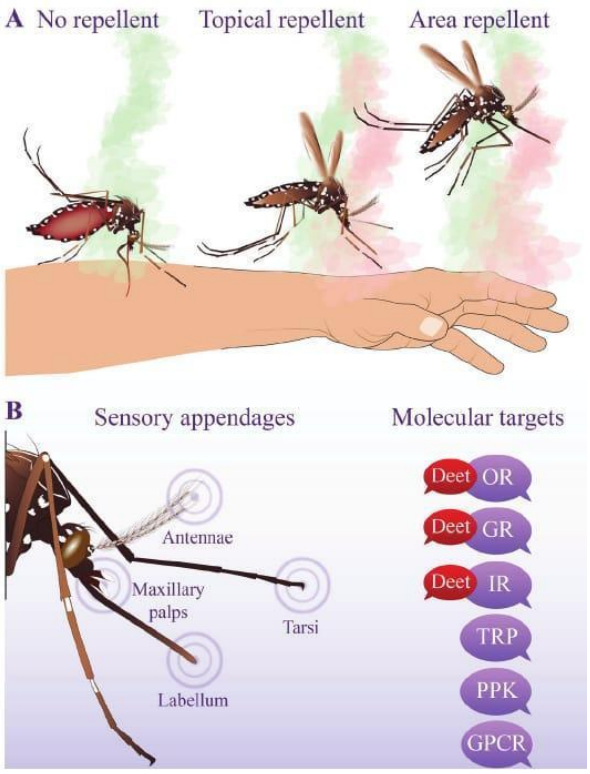
Mosquito Repellent Clothing: Permethrin-treated clothing.
Ultrasonic Repellents: Ultrasonic devices.

- **Synthetic Mosquito repellent:-**

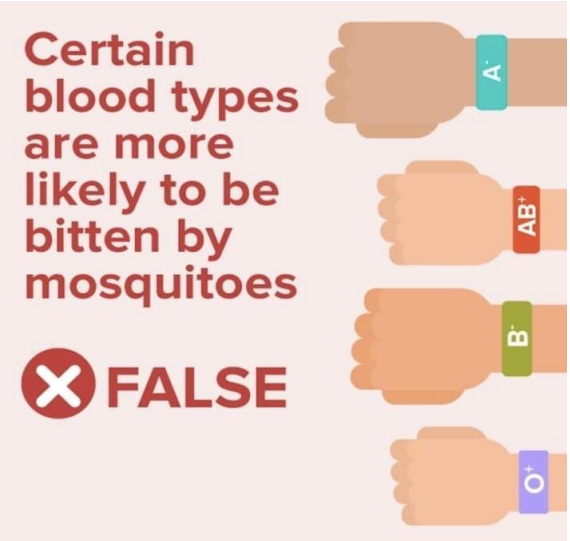
- The majority of commercial repellents are made using such as N, N- diethly-meta toluamide (DEET), allethrin N, N-diethyl Mendelian acid amide and dimethyl phthalate.
- However, it has been determined that chemical repellents are not safe for public Health.
- Utilizing traditional plant knowledge to develop natural repellents as an alternative to chemical- based repellents is highly valuable.

- **Mechanism of Action of Mosquito Repellence**

- Often mosquito repellents work by either masking Human odor or by using smell that insect's like and Instinctively avoid.
- olfactory receptors (ORs) and gustatory receptors (GRs) are targeted repellents for mosquitoes.in order to find the their hosts.
- Mosquitoes use their olfactory system to detect carbon dioxide (CO_2) and other human scents.
- Heat sensors are used by mosquitoes to identify body heat emitted by warm-blooded hosts.
- In order to prevent landing and biting, Repellents acting as contact irritants.



- **Mosquitoes prefer o+ People (myth or fact)**



1.One study published in the Journal of medical Entomology in 1972 suggested that mosquitoes were more attracted to individuals with Type O blood.

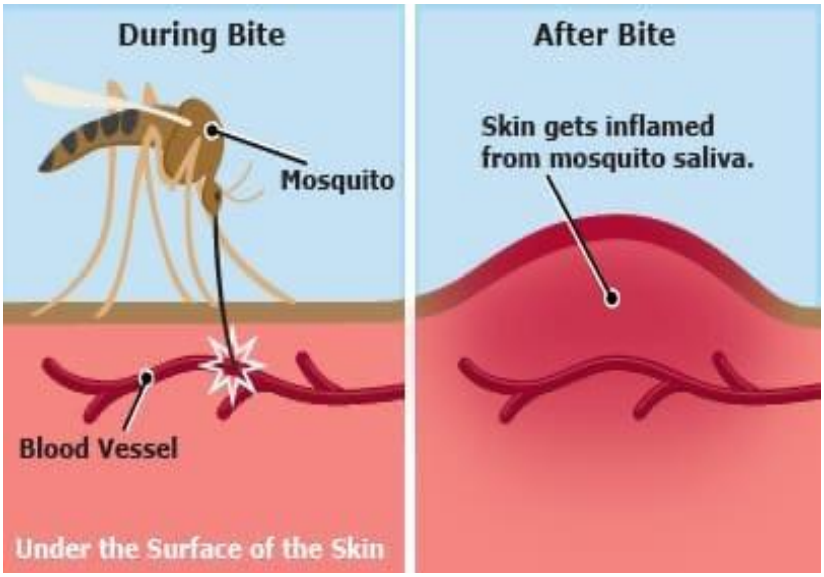
2.Another study published in the same journal in 2004 Found no significant difference in Mosquito attraction between individuals with different blood types (A,B,AB,O)

3. It's essential to note that mosquito attraction is influenced by a complex array of factors, including:-

•Carbon dioxide emission
•Body temperature
• Moisture
• Visual cues
•Volatile organic compounds emitted by the skin.

4.while blood type might play a minor role in mosquito attraction, it is unlikely to be a significant factor. So if you're O positive individual, you're not necessarily more likely to attract mosquitoes!.

- **Layer of skin affected by Mosquito: -**



- The layer of skin that is highly affected by mosquitoes is the Dermis. The dermis is the second layer of skin.
- When a mosquito bites, it's saliva and other substances enter the dermis, triggering an immune response and leading to the release of histamine and other chemical mediators.
- This can cause blood vessels in the dermis to dilate, leading to increased blood flow, swelling and inflammation[5].

II) Drug Profile

1. Cymbopogon nardus (Citronella)

Synonyms	Citronella grass, Nardus.
Family	Poaceae.
Biological Source	It is the oil obtained by the steam distillation of fresh leaves of Cymbopogon nardus (L.)Rendle,
Geographical Source	Citronella is native to Southeast Asia and grown commercially in Sri Lanka, India, Burma, Indonesia.
Cultivation and Collection	It is spread through seeds. It may not withstand cool, wet winters and requires a long, warm season. They are planted with 2,000 to 3,000 above sea level during the summer.
Characteristics	It is tall tufted perennial grass with narrow leaf blades that forms a clumps in tropical environment. They reach a height of 5-6 feet. The flat greyish- green leaves measures roughly 3 feet in lenth and 1 inch in width.
Chemical Constituents	The main chemical components of citronella oil are citronellic acid, geran-iol, nerol, citral, borneol, camphene, citronellol, citronellal, dipentene, and limone
Uses	Perfumery, Insect Repellent, Insecticide, Parasitic.

2. Eucalyptus globules.

Synonyms	Dinkum oil, lemon gum tree, Blue gum tree
Family	myrtaceace
Biological Source	Eucalyptus oil is the volatile oil obtained by the hydrodistillation of fresh leaves of eucalyptus globulus.
Geographical source	Native to southeastern Australia
Characteristics	Trichomes are absent, Presence of sunken Stomata, Epidermis is polygonal, Presence of schizogenous oil glands
Chemical Constituents	Cineole (eucalyptol, 70-85%), Terpenes, Polyphenolic acid caffeic acid, Flavanoids Eucalyptin, Rutin.
Uses	Antimicrobial, Flavouring agent, Expectorant, mask human scent

3.Ocimum sanctum (Tulsi)

synonyms	sacred basil
Family	Labiatae
Biological Source	Tulsi consists of fresh and dried leaves of ocimum sanctum.
Geographical Source	Branched annual plant found throughout India. It is considered as sacred Hindus.
characteristics	It is much branched small herb and 30 to 75 cm in height.
chemical constituents	Eugenol, rosmarinic acid, linalool, and flavonoids.
Uses	Antibacterial, insecticidal, stimulant, aromatic, spas molytic, natural mosquito repellent.

4. Syzygium aromaticum (Clove)

Synonyms	Clove buds, clove flowers.
Family	Myrtaceace
Biological Source	Clove consists of the dried flower buds of Eugenia Caryophyllus Thumb.
Geographical Source	Clove tree is a native of Indonesia. It is cultivated mainly in Islands of Zanzibar, Pemba,Brazil,Amboiana and sumatra
Characteristics	Clove is reddish-brown in colour, an upper crown and a hypanthium.The hypanthium is 10 to 13 mm long.
Chemical Constituents	eugenol, eugenyl acetate, B-caryophyllene, flavonoids, phenolic acid.
Uses	kills intestinal parasites antimicobial

5. Azadirachta Indica (Neem)

Synonyms	Nimtree or Indian lilac.
Family	Meliaceae
Biological Source	Azadirachta Indica tree, The leaves, seeds, fruit.
Geographical Source	It grows in tropical and semi tropical regions. Widely found in Burma, India and Pakistan. This is a very fast growing, ever green tree which reaches the height of 15 to 20 meters.
Characteristics	very fast growing, ever green Tree.
Chemical Constituents	Neem tree has numerous medicinal properties by virtue of its chemical compounds. Seeds of the Neem tree contain the highest concentration of Azadirachtin. Apart from Azadirachtin salannin, gedunin, azadirone, nimbin, nimbidine, nimbicidine, nimbinol, etc are other important liminoids of neem.
Uses	protect grains and cereals from pests, insecticide, natural pesticides.

6.Citrus Sinesis (sweet orange)

Synonyms	Citrus vulgaris, Citrus Bigaradia, Seville orange peel.
Family	Rutaceae.
Biological Source	Vitex negundo is an erect shrub or small tree growing from 2 to 8 m (6.6 to 26.2 ft) in height. The bark is reddish brown.
Geographical Source	It is mainly cultivated in India, china, Spain.

Characteristics	It is a small tree with a smooth, greyish brown bark.
Chemical Constituents	1 to 2.5%volatile oil. The principle component of volatile oil is 90% limonene and aldehydes citral, citronellal, amorphous aurantiamarin and hesperidin, isohesperidin, vitamin C, and Pectin.
Uses	Aromatic, Stomachic, carminative and flavouring agent[6].

III) Literature Review:

Citronella:

*Ansari et al. [2005] Researchers have explored the repellent properties of citronella, an essential oil derived from lemongrass (Cymbopogon spp.). One study revealed that citronella extracted from lemongrass demonstrated a 100% repellent effect against Anopheles culicifacies mosquitoes for up to 11 hours. *Tawatsin et al. [2010] found that citronella oil repelled Anopheles stephensi and Anopheles dirus mosquitoes for 8 and 6 hours, respectively. Additionally, studies showed that citronella essential oil provided complete protection against Anopheles minimus for 2.16 and 0.8 hours, depending on the concentration. The repellency of citronella against various Anopheles species ranged from 52% to 85%, varying with concentration and species.

Eucalyptus:

* Amer et al. [2015] Eucalyptus, a widely cultivated tropical plant, has been studied for its repellent properties. Five studies examined various eucalyptus subspecies and found that narrow-leaved, lemon-scented, and broad-leaved eucalyptus provided protection against Anopheles stephensi mosquitoes for 8 hours, while Eucalyptus globulus offered protection for 5.5 Hours. *Auysawasdi et al. [2016] A study tested Eucalyptus globulus essential oil at various concentrations (5-25%) against Anopheles dirus mosquitoes. All concentrations provided complete repellency, lasting between 1.7 and 3.4 hours, depending on the concentration. Additionally, a 0.1 ml dose of Eucalyptus globulus repelled Anopheles dirus for 1.58 hours, while 100 µl of Eucalyptus citriodora repelled Anopheles minimus for 0.5 hours.

Clove (Syzygium aromaticum):

Phasomkusolsil Sr al. [2011] Clove, natural spice, has been found to have various health benefits. Researchers investigated its repellent effect against Anopheles mosquitoes in six studies. The results showed that clove, at concentrations of 0.02, 0.10, and 0.21 mg/cm2, demonstrated a dose-dependent repellency of 82%, 92%, and 98% against Anopheles dirus mosquitoes.

Orange Oil (Citrus sinesis):

*Murugan et al. [2012] Orange, a subtropical citrus plant, has been studied for its repellent properties against Anopheles mosquitoes. Four studies investigated the effect of orange extract on Anopheles species. The results showed that orange extract concentrations of 50-450 ppm provided complete protection against Anopheles stephensi, with repellency times ranging from 0 to 2 hours, and 100% repellency achieved at certain concentrations

Neem (Azadirachta Indica):

Amer et al. [2006]. Neem, a versatile tree native to tropical India, has been studied for its repellent properties against Anopheles mosquitoes. Two studies investigated Neem's effect on Anopheles species. The results showed that 20% Neem oil provided a mean repellency of 71% and complete

protection for 3 hours against Anopheles arabiensis in a field trial.

Plan of Work:

*This systematic review examined the repellent effects of plant-based substances on Anopheles mosquitoes, which transmit malaria. Researchers searched electronic databases, including PubMed and Web of Science, for eligible studies published between 1999 and 2018., investigate plant extracts or essential oils, and report repellency percentages or complete protection times against Anopheles mosquitoes.
*Data was collected from each study using a standardized form to ensure accuracy and critical appraisal.
*Additionally, a cross-sectional survey was conducted among 319 participants, who answered a 10-question questionnaire. The survey was administered online between March 5 and April[7].

IV) MATERIALS AND METHODOLOGY:

Formulation of mosquito repellent spray:

Ingredients	Quantity	Use
Citronella	5.00 mL	Strong Repellent
Eucalyptus essential oil	5.00 ml	Antiseptic, repellent
Tulsi essential oil	1.00 ml	Antibacterial
Clove oil	3.00 ml	Insecticidal properties
Sweet orange oil	2.00 mL	Aromatic, flavoring
Neem extract	6.00 ml	Insecticide
N- Hexane	6.00 ml	solvent
Ethanol	50 ml	Dissolve active
Tween 80	10 ml	Emulsifier
Distilled water	qs	

Formulation of mosquito repellent Candle:

Preparation of Azadirachta Indica (Neem) seed extract:

Put the neem seed powder into a thimble.

⬇

Put thethimble in the soxhlet chamber

⬇

Fill a round bottom flask with a solvent like n-Hexane.

⬇

Set up the flask for Soxhlet extractor

⬇

Heat the solvent using the Heating mantle until it reaches it's boiling point.

⬇

Continue extraction process for 6-12 hrs.

⬇

After extraction is complete, collect extract from round bottom flask.

⬇

Filter the extract using filter paper and evaporate the solvent using water bath and allow to air dry.

⬇

Get the solvent - free oil.

- **Preparation of Syzygium aromaticum (Clove) oil:**

Put dried clove buds in steam flask.

⬇

Add water and boiling chips to flask

⬇

Set up a distillation apparatus with a condenser and
a receiving flask.

⬇

Turn on water and heat.

⬇

Collect the distillate to room temperature.

⬇

Transfer distillate to separately funnel.

⬇

Separate clove oil from water through decantation[8].

- **Procedure of mosquito repellent Spray:**

Mosquito repellent Spray

In beaker 1 combine the essentials Oils in given quantity and mix well to create a uniform blend.

⬇

In beaker 2 mix Ethanol (50mol) with Tween 80 (10 ml) and stir well to create uniform blend.

⬇

Combine the essential oil blend to Ethanol - Twen 80 solution and mix well to combine.

⬇

Add distilled water to the mixture and mix well.

⬇

Filter the mixture through a cheesecloth into a clean container.

⬇

Fill spray bottle with filtered mixture.

⬇

Label and store in cool, dark place.

- **Procedure of mosquito repellent candle:**

Mosquito Repellent Candle

Take beeswax and weigh accurately

⬇

Melt the beeswax in beaker with help of heating mantle until completely melted

⬇

After properly melting start combining the essential oils and extract

⬇

Weigh required amount of each essential oil and extract and add to the melted beeswax, Stir the mixture thoroughly

⬇

Continue heating the mixture for a few minutes to ensure everything is well combined

⬇

Add colorants and stir well

⬇

Pour above mixture in suitable size mould

⬇

Allow mould to cool at room temperature

⬇

After ½ hr remove the candle from mould[9]

V) Evaluation Parameters:

- **Evaluation of mosquito repellent spray**

1] Physical appearance

• **Colour-** Yellowish orange
• **Odour-** Aromatic.

2] pH

The pH of the liquid spray was measured using a pH meter after diluting the liquid with distilled water. The pH was found to be 5.09 at 25°C.

3] Surface layer uniformity

To determine whether the essential oils in the formulation formed a layer on the surface of water. To conduct this test, contaminated water was placed in a beaker, and the spray solution was added to the surface. The surface was then observed to determine if a layer formed on the water's surface.

4] Skin irritancy

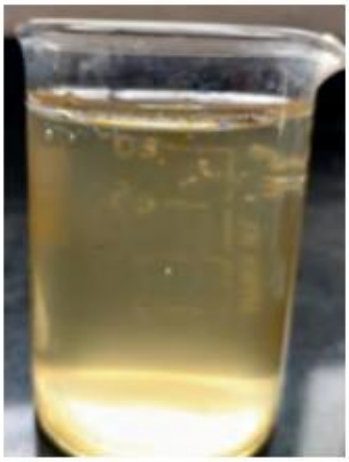
The solution was sprayed on the skin and checked for irritation every hour. It was found to be non-irritating and was absorbed quickly. No redness or irritation was observed on the skin

5] Mosquito repellency test

- Mosquito repellency test was done by simply selecting the mosquito-prone areas like bushes, shrubs, laboratory corners and canteen
- Then divide test subjects into 2 groups: a treated group and an untreated (control) group
- Apply mosquito repellent spray to treated group
- Both group stand or sit in test areas for 1hr
- Remarks were noted down and checking if the mosquitoes are sitting on skin or escaping away[10,11].

Sr. No.	Areas	Remarks
1	Bushes	Mosquitoes repelled
2	Laboratory corners	Mosquitoes repelled
3	Canteens	Mosquitoes repelled
4	Garden	Mosquitoes repelled

PH Meter



Surface layer uniformity

No Skin Irritancy

- **Evaluation of mosquito repellent candle**

1] Organoleptic test:

Colour: colour of formulation was found to be Yellow

Fragrance: Fragrance of formulation was found to be satisfied.

Texture: Formulation texture was found uniform.

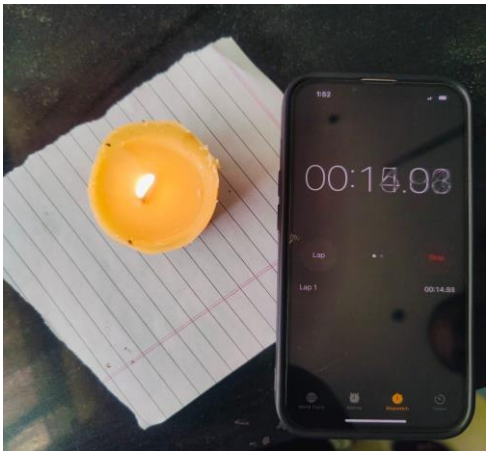
2] Uniformity of mass: 2 candles were selected and weighed individually. The weight of individual candle was noted. Average weight was calculated, and the individual Weights were compared with the average weight. Average weight was observed 43g.

Candle No.	Weight of Individual Candle	Length (cm)	Width (cm)
C1	43.6 gm	3.3 cm	4.5 cm
C2	42.6gm	3.3 cm	4.5 cm

3] Irritancy: Irritancy factors include teary eyes and irritating cough. Candles were lighted to evaluate above factors. No teary eyes and cough

4] Emissions: Emission factors include soot production and fragrance emissions. Mild soot was produced but has pleasant odour.

5] Burning time: Burning time visualized using 2 candles named as C1 and C2 respectively their Burning time is calculated using watch.



Candle No	Burning Time	Application Time	Efficiency
C1	35 min	6-7 pm	Good
C2	40 min	6-7 pm	Good

6] Flammable Test: The prepared candle was tested for flammability to explore mosquito repelling habits and burned Quality with respect to burning tine and subsequently it's spotting process effectiveness flammability test for candle was conducted to verify its apparent flammability in the Laboratory.

7] Melting Point: The melting point was determined by the maximum temperature that allowed the changing of state of the candle. For this small quantity of sample were put in a

capillary tube. The temperature was set at 50 degree Celsius, Temperature was increased until a change in state was observed and the temperature noted. The melting point of herbal mosquito repellent was found to be 60-65 degree celcius[12,13].

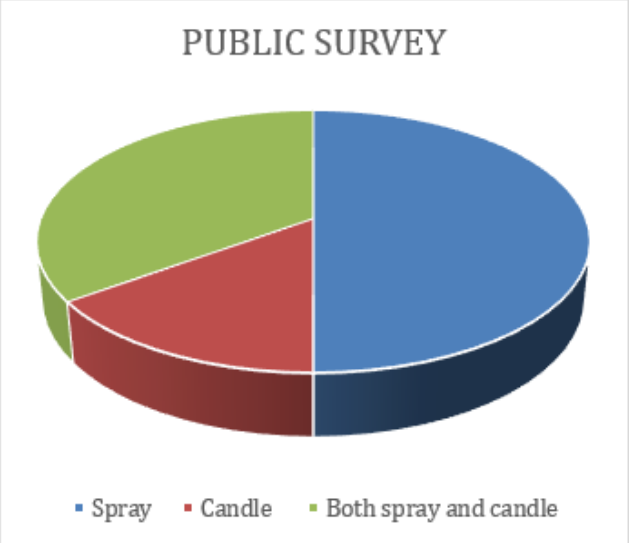
Public Survey:

- Public survey was done to study safety and efficacy of both formulations and also which form is more effective.
- A questionnaire comprising 10 Questions on mosquito repellent spray and 10 questions on mosquito repellent candles was given to participants through Google form link.
- About participants took part in a survey and formulation was distributed to them and feedback was collected.
- The data collected was studied and represented statistically.

VI) RESULT AND DISCUSSION

Result on basis of survey:

- From the survey conducted, it was found that 10 participants have give positive answers to the spray form of repellent, 7 participants give response to both formulation and 3 participants give response to candle formulation.
- It is seen that more people are in favour of using herbal mosquito repellent spray.



VII) CONCLUSION

The mosquito repellent spray demonstrated 100% mosquito repellency. However, the effectiveness of plant-based repellents can vary depending on factors like: -

- ❑ Mosquito species
- ❑ Quality of plant extracts and essential oils
- ❑ Volunteer characteristics (age, sex, biochemical attractiveness)

- The herbal mosquito repellent candle is made from natural ingredients and causes no skin irritation or allergic reactions.
- The candle is lightweight, easy to use, and beneficial for both mosquito repellency and human health. The used herbs and essential oils are safe and effective as mosquito repellents.

A comparative study of mosquito repellent sprays and candles generally concludes that spray repellents are significantly more effective at

repelling mosquitoes than mosquito repellent candles, particularly when considering their coverage area and duration of protection; while candles may offer some limited protection in close proximity, their effectiveness diminishes rapidly with distance, making them unsuitable for large areas or extended periods of outdoor activity[14,15].

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