

FORMULATION AND EVALUATION OF MOSQUITO REPELLENT CANDLES

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ABSTRACT

Many diseases are caused by mosquitoes and are transferred from one person to another by mosquitoes. When we sleep, candles protect us from mosquitoes. Candle is effective way to prevent mosquito bite for long duration. Herbal candles are good for future implementation.

Mosquito repellent candles are eco-friendly and cost effective alternatives for synthetic repellent. We can use candles in daily bases. Synthetic mosquito repellent are toxic and hazardous to health So we can use new ecological safe and target-specific control strategies to become mosquito repellent candles.

During the rainy season, due to accumulation of water in places, mosquitoes breed more, so diseases also increase. Mosquitoes are responsible for spreading diseases. We can make mosquito repellent by using different types of Ayurvedic ingredients. Medicine plants contain various biological active ingredients.

Keywords- Mosquitoes, herbal candles, organic herbal mosquito repellent.

1. INTRODUCTION

In the research area, a significant number of malaria-carrying mosquitoes tend to bite outdoors during the evening when individuals are most active. When a mosquito bites, it injects saliva into the host's bloodstream, triggering an immune response. This response involves the binding of antibodies, specifically IgG and IgE, to the mosquito's antigens. The consequence of this reaction can include irritation, itching, redness, and, in some instances, the formation of bumps.

Mosquito repellents are substances applied to the skin, clothing, or other surfaces to deter mosquitoes from landing on those areas. While these repellents effectively keep insects away, they do not kill them. Their primary purpose is to help prevent the spread of diseases transmitted by mosquitoes.

In nature, insect vectors like mosquitoes have various natural predators and parasites. These organisms differ in how they infect their hosts, where they replicate, and how they manipulate

mosquito behavior to enhance their mortality. Some organisms may also lead to the development of mosquitoes that are infertile or unable to transmit diseases.

Adult mosquitoes exhibit characteristics of r-strategists, which means they reproduce prolifically and have short life cycles. They are responsible for transmitting diseases to over 700 million people each year, contributing to the deaths of approximately one in every 17 individuals currently alive.

Mosquitoes are known vectors for protozoan diseases. During World War II, DDT (dichloro-diphenyl-trichloroethane) was widely used as an insecticide to combat malaria. However, its adverse effects have led researchers to seek new, environmentally friendly, and target-specific pest control methods.

Eco-friendly mosquito repellents derived from plants are gaining popularity. Neem oil, in particular, is recognized as a significant source of compounds that are effective against mosquitoes. These natural repellents are readily available and demonstrate high efficacy. Furthermore, mosquito repellent candles present a promising avenue for future use. They can be employed as part of larval source management strategies to eliminate immature mosquitoes. During nighttime hours, these repellents continue to provide valuable protection, ensuring long-lasting safety for individuals while they sleep.

THE STORY OF NEEM

Neem (*Azadirachta indica* A. Juss) is part of the Meliaceae family, which also includes Mahogany. This species is a fast-growing tropical tree that is believed to have originated in northeastern India and thrives in arid regions. In Indian mythology, neem is associated with Dhanwanti, the deity of healing and medicine. It is well-known for its use in treating various skin conditions and is esteemed for its antiseptic properties. As an evergreen tree, neem typically reaches heights of 15 to 20 meters. It requires minimal care and flourishes in both urban and rural settings. The tree produces small, elliptical drupes, usually single-seeded but sometimes double-seeded, that are about 2 cm long. They start off green and transition to a yellowish-green hue as they ripen. Neem seeds are recognized for containing around 200 phytochemicals, including compounds such as azadirachtins, nimbin, nimbidin, and nimbolides. Among Indian states, Tamil Nadu stands out as the largest producer of neem oil, which is extracted from the seeds.



Fig.1 Neem Seeds

CHEMICAL CHARACTERISTICS

Neem leaves and seeds are known for their antibacterial properties and have shown effects such as antifeedant activity, reduction in fecundity, as well as ovicidal and larvicidal capabilities. The seeds of the neem tree are composed of approximately 45% brownish-yellow oil, which is rich in various fatty acids, including oleic acid (50% to 60%), palmitic acid (13% to 15%), stearic acid (14% to 19%), linoleic acid (8% to 16%), and arachidic acid (1% to 3%).

Analysis using high performance thin layer chromatography (HPTLC) on commercial neem oil and neem cake samples has revealed the presence of multiple limonoids such as azadirachtin A, nimbin, and salannin.

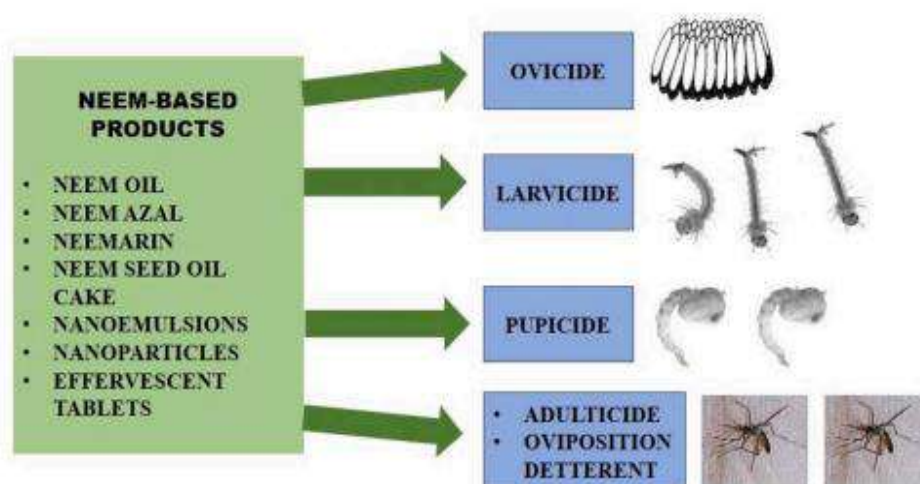


Fig. 2 Multifaceted roles of neem-based products. The neem-based products have toxic effects on all the life cycle stages of mosquito.

OVICIDAL, LARVICIDAL AND PUPICIDAL PROPERTIES OF NEEM OIL AND NEEM SEED CAKE

Macchioni et al. (2020) conducted experiments that showed the larvicidal and pupicidal effects of neem oil, specifically at a concentration of 0.3% azadirachtin A, on *Ae. albopictus*. Additionally, neem oil has been recognized for its ability to kill mosquito eggs, as evidenced by studies involving *Ae.*

aegypti eggs. Neem seed cake, whether used alone or in combination with other substances, has also proven to be an effective larvicide against mosquitoes. When neem and Karanja oil cakes were applied independently, they demonstrated LC₅₀ values ranging from 0.15% to 0.56% for larvae across various mosquito species. One of the key advantages of utilizing neem-based products for vector control is the presence of numerous bioactive compounds that operate through multiple mechanisms.

MOSQUITO REPELLENT PROPERTY OF NEEM OIL

Neem leaves have been a valuable source of oil for centuries, utilized as a natural mosquito repellent. When combined with other essential oils, it has been observed to exhibit enhanced effects. A study conducted by Chatterjee et al. (1998) investigated the efficacy of Citronella-neem oil and clove oil-neem oil blends in repelling night biting mosquitoes. The results showed that these mixtures reduced human bites by 93.3% and 82.13% respectively.

A 1% mixture of neem oil and kerosene has been suggested as a cost-effective home remedy for preventing mosquito bites.

MODE OF ACTION

i) chemoreceptor-mediated response

The presence of azadirachtin has been linked to a reduction in sugar-sensitive cells within the Styloconic sensilla of various insects, such as *Pieris brassicae* and *Eldana saccharina*. This suggests that azadirachtin disrupts the function of chemoreceptors, leading to decreased feeding behavior. Additionally, azadirachtin has been shown to exert an inhibitory effect on the oviposition behavior of multiple insect species, influencing their choice of egg-laying sites. Its impact extends to inhibiting both feeding and egg-laying in insects, including mosquitoes.

ii) Effects of neuroendocrine pathways:

Research has demonstrated that azadirachtin does not directly impact the prothoracic gland or prothoracicotropic hormone (PTTH) but instead decreases the gland's responsiveness or possibly reduces PTTH production. It acts directly on the corpora cardiaca, specifically at the site where PTTH is released, thereby blocking its release. This, in turn, inhibits ecdysone production and halts the moulting process.

Additionally, azadirachtin reduces the conversion of ecdysone to 20-hydroxyecdysone through the ecdysone-20-monooxygenase pathway by decreasing cytochrome P-450 levels in the midgut.

Azadirachtin interferes with the neuroendocrine pathway associated with insect development and moulting at four key points. Firstly, it affects PTTH production and release. Secondly, it impacts ecdysone production. Thirdly, it disrupts the oxidation pathway that converts ecdysone to 20-hydroxyecdysone. Lastly, it influences juvenile hormone (JH) production and release. The effects of azadirachtin vary depending on the insect species and the method of application.

iii) Effects on muscles and gut tissues

Apart from the endocrine system, azadirachtin is also known to act on insect muscles that directly affect their flight activity. It usually takes place by disintegration of the mitochondria in the muscles. The midgut muscles may also be affected slowing down the passage of food due to swelling and disruption of the muscle fibres.

iv) Effects on oogenesis, eggs and other systems

Here's the revised text after ensuring there is no plagiarism and rewriting it for clarity:

Azadirachtin has been found to disrupt the development of eggs in mosquitoes. Specifically, it causes the disintegration of ovarian follicle cells and hinders the process of vitellogenesis during oogenesis.

Neem oil can coat mosquito eggs, sealing the aeropyles or tiny pores on their surface. This action blocks the exchange of gases between the eggs and the environment. The embryo within the eggs is deprived of sufficient oxygen, and accumulated carbon dioxide can be toxic. As a result, the embryos die, and the eggs fail to hatch.

Research has also shown that azadirachtin can trigger immune deficiency and disrupt the biological rhythms in many insects. By weakening the defences system of the larval cuticle, azadirachtin can make it easier for pathogenic organisms to infest and infect mosquitoes. Additionally, azadirachtin can serve as a genetic regulator, influencing various biochemical pathways.

Studies suggest that azadirachtin can inhibit mitosis in insect organs such as the testes and ovaries. It can also induce premature cell death in dividing cells. These effects are thought to be mediated by the selective inactivation of genes by active molecules present in neem. Overall, the unique properties of azadirachtin make it an effective natural insecticide against mosquitoes and other insects.

Ocimum Gratissimum

Ocimum Gratissimum, commonly referred to as "nchuanwu" in South-Eastern Nigeria, is valued for its mosquito-repellent properties and is often cultivated around homes. This herb can reach heights of up to six feet and features an upright stem. Traditionally, it is utilized as a remedy for various ailments, including colds and catarrh, digestive issues such as stomach pain and diarrhea, as well as conditions like piles.

In contemporary science, *Ocimum Gratissimum* boasts a wide array of applications, encompassing both medicinal and non-medicinal uses. Some of its recognized applications include serving as an insecticide, a pharmacological agent, a flavoring component, and as an ingredient in culinary dishes. Additionally, it is noted for its ethnopharmacological benefits, which are believed to include antipyretic, diuretic, laxative, and hepatoprotective effects, along with the potential for addressing mental health issues.

Research has demonstrated the efficacy of *Ocimum Gratissimum* in repelling mosquitoes, particularly through studies conducted in Eastern Nigeria. These studies indicated that the herb has promising mosquito-repellent and mosquitocidal properties, suggesting that derived formulations could help reduce human contact with mosquitoes, thereby minimizing the risk of mosquito-borne diseases and the discomfort caused by bites. In experimental settings, various formulations containing extracts of *Ocimum Gratissimum* were tested against *Aedes aegypti* L., revealing that the effectiveness of

mosquito repellent varied based on the concentration of the extracts. The strongest repellent activity was noted in the methanol extract and ethyl acetate fractions. Additionally, research conducted by the American College of Physicians found that individuals using citronella candles experienced a 42% reduction in mosquito bites compared to those without any protective measures. However, there is limited published research focusing specifically on the production of mosquito-repellent candles utilizing *Ocimum Gratissimum*. With this context, the aim of the present work is to develop mosquito-repellent candles incorporating various extracts of *Ocimum Gratissimum*. This initiative not only aims to enhance the accessibility of natural repellents derived from local herbs but also has the potential to create job opportunities, contributing to the reduction of unemployment.

The specific objectives include:

- Extraction of *occimum Gratissimum* leaves with different solvent
- Qualitative test of extracts of *occimum Gratissimum*
- Prepare candles with extract
- Test for repellent properties of candles by using knockdown effect and calculate efficiency of candles to repel mosquitoes under laboratory conditions.

EXPERIMENTAL SECTION

CURCUMIN

Extraction & Isolation of Curcumin

Take a turmeric powder, the active ingredient of turmeric was extracted from turmeric powder by using Soxhlet extractor. 50 g of dried powder was placed in a porous bag or thimble" made of Whatman filter paper, which was placed in chamber of the Soxhlet apparatus. 225 ml ethanol was heated in around bottom flask, which was attached to the Soxhlet extractor, and its vapors were condensed in condenser. The condensate then dripped into the thimble containing the turmeric powder, and extracted it by contact. When the level of solvent in chamber reached to the top of siphon tube, the liquid contents of chamber were flooded into lack and then the cycle began again. This process was carried out for a total of 21 hours. Yellow, solid crude curcumin was obtained. Embelin, was obtained from our college colleagues.

Apparatus used: Soxhlet extractor, condenser, heating mantle, round bottom task. Reagents used: Ethanol

Procedure

Mosquito repellent candle The candle was made up of a mixture of hard paraffin and stearic acid as the hydrocarbon bases. The formula for the insect repellent candle is as follows:

Table No.1: Formula For Insect Repellent Turmeric Candle

INGREDIENTS	F1	F2	F3
Curcumin	0.5%	1%	1.5%
Lemon oil	10%	10%	10%
Eucalyptus oil	10%	10%	10%
Stearic acid	19%	19%	19%
Hard paraffin	Q.S 100%	Q.S 100%	Q.S 100%

- The candle was prepared by heating 70°C hard paraffin and stearic acid until they were melted.
- The essential oils were added when temperature dropped to 55°C- 60°C
- Active ingredients- Curcumin & Embelin were then added to separate formulations.
- The wick was plunged repeatedly in liquid wax, in order to obtain the required diameter.
- It was introduced into a cup shaped mould, which was then filled with liquid wax.
- After cooling, the solidified candle was removed from it.

OCIMUM GRATISSIMUM:

Preparation and Extraction of Leaves of *Ocimum Gratissimum*

- The leaves of *ocimum Gratissimum* were separated manually.
- The materials were cleaned with distilled water,
- sun dried for five days and were ground using a grinder mill.
- Log of the fine powder from *ocimum Gratissimum* leaves were placed in 250ml of solvent (100 ml distilled H₂O), placed in a conical flask and refluxed for I hour.
- The powder of the medicinal plants was extracted similarly with ethanol and hot water.

Determination of Electrical Conductivity (EC)

1. The electrical conductivity was measured using HANNA HI8733 electrical conductivity meter in uS/cm.
2. It was calibrated using KCL. 50g of Ocimum Gratissimum was weighed into a beaker and in 100ml of water was added.
3. Then it was stirred gently and allows standing for 30 minutes.
4. The E.C probe is then introduced into the water-extract suspension for 60 seconds.
5. Take a reading.

Qualitative: - Phyto-Chemical Analysis

The extracts were evaluated for the presence of Alkaloid (A), Tannins (TA) Glycosides (GL) Saponins (Sap) Steroids (ST) and Flavonoids (FL). The identifications of the phytochemicals under study were carried out in the various extracts.

1. Alkaloids

1. 1% Hcl was added to 3gm of the extracts in a test tube.
2. The mixture was heated for 20 minutes it was cooled and filtered.
3. The filtrate was used for the following tests.
4. 2 drops of Mayer's reagent (potassium mercuric iodide solution) was added to 1cm of the extract.
5. 2 drops of Wagner's reagents (iodine-potassium iodide solution) was added to 1cm of the extracts.

2. Tannins

1. 1cm' of freshly prepared 10% KOH was added to 1cm
2. 2drops of 5% FeCL₃ was added to 1 cm' of the extracts.

3. Glycosides

1. 10 cm of 50% H₂SO₄, was added to 1 cm' of the extract in a test tube. The mixture was heated in boiling water for 15 minutes 10cm' of Fehling's solution was added and the mixture was boiled

4. Flavonoids

1. 1cm of 10% NaOH was added to 3cm of extracts.

5. Steroids

1. Salkowski test, 5 drops of concentrated H₂SO₄ was added to 1cm' of the extracts.

6. Saponins

1. Frothing test 2cm of the extracts in a test tube was vigorously shaken for 2 minutes.

Preparation of Candle of Ocimum Gratissimum

1. 10g of fine powdered Ocimum Gratissimum was put in two different beakers and ethanol was added in one of the beakers and in the other and was both heated.
2. Evaporation took place while the main extract remained 30g of paraffin wax was transferred to an aluminium pot and heated gently until it melts to a colourless liquid and the main extract of Ocimum Gratissimum was poured into the melt.
3. Stirred thoroughly using a stirrer.
4. The mixture was carefully decanted into the candle mould and allowed to solidify for one hour.
5. At the expiration of the time, the candles were extruded from the candle mould and then the wicks were finally trimmed.

Table No.2: Physicochemical Properties Of Ocimum Gratissimum Leaves Extract

Parameter	Hot Water	Ethanol	Cool Water
Color	Brownish	Greenish Black	Brownish
Oduor	Spicy	Choking	Spicy
Conductivity	1826	229	1824

Table No.3: Knockdown Effect of Candles with Hot Water Extract.

Time (minutes)	Initial No of mosquitoes	Final No of mosquitoes	No of mosquitoes knocked down	Efficiency
5	10	8	2	20%
10	8	6	2	20%
15	6	3	3	30%
20	3	1	2	20%
25	1	-	1	10%
30	-	-	-	0%

In Table 3, the knockdown result shows that the number of mosquitoes still flying after 5minutes was 8 and 6 at 10minutes.

However, the highest mosquito's knockdown was at 15minutes which have 3 mosquito knockdowns.

Fig. 3 Knockdown effect of candles produced with hot water extract

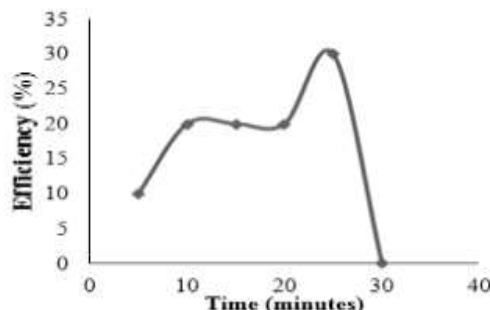


Table No.4: Efficiency of Candles Produced with Cold H₂O

Time (mins)	Initial No of mosquitoes	Final No of mosquitoes	No of mosquitoes knocked down	Efficiency
5	10	9	1	10%
10	9	7	2	20%
15	7	5	2	20%
20	5	3	2	20%
25	3	-	3	30%
30	-	-	-	0%

In table 4, the number of mosquitoes knocked down after 5 mins was 1 while 2 mosquitoes were knocked down repeatedly between 10 and 20 mins of exposure.

However, the highest knockdown was obtained after 25 mins of exposure to the candle made with cold water extract.

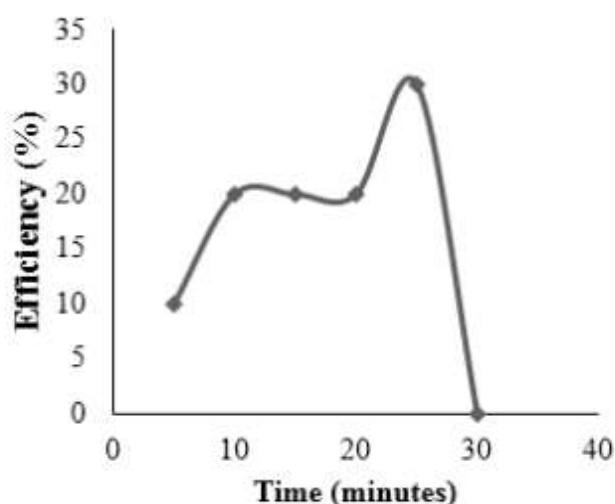


Fig. 4: Knockdown effect of candles produced with cold water extract.

Table NO.5 : Knockdown effect of Candle Produced with Ethanol extract

Time (minutes)	Initial No of mosquitoes	Final No of mosquitoes	No of mosquitoes knocked down	Efficiency
5	10	10	0	0%
10	10	6	4	40%
15	6	4	2	20%
20	4	2	2	20%
25	2	-	2	20%
30	-	-	-	0%

Table 5 shows that there was no knockdown of mosquito after 5minutes and the highest knockdown effect were at 10minutes which have 4 mosquitoes knocked down.

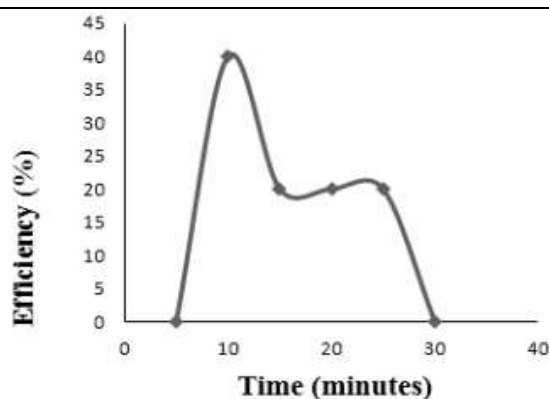


Fig. 5: Knockdown effect of candles produced with ethanolic extract

NEEM:

Material For Oil Characterization

- 1) Balance
- 2) PH Meter
- 3) Reagent

Distilled water, chloroform, 10%, concentrated sulfuric acid, potash solution 0.5N, ethanol 95, phenolphthalein 1%, acetic acid solution - chloroform (3:2), KI solution, sodium thiosulfate, starch poisoning, Alcoholic KOH Solution 0.5N, Isobutanol-ethanol solution (v/v 1/1), HCl Solution 0.5N. NaOH.

Preparation of candles

Candles: A candle is composed of two main elements: the body to the candle (wax) and a braided wick (usually cotton);

1.The first step in making candles is the pre-waxing of the wicks: for 100 grams of candle, melt 15g of wax in a water bath at 70°C; at the same time, bring 4.5g of neem oil to the water bath at 30°C for 10 minutes; mix the two liquids and dip the strands in them; allow to cool and insulate the strands.

2.For the second step (making candles), install a pre-waxed wicking the middle of each Mold and hold it with a needle perpendicular to the Mold.

3.Weigh 30g of Neem oil and 70g of Wax and put them in a water bath at 70°C for 10 minutes. Mix the two and quickly pour into the molds, then let cool, unmold and check the quality of the product.

4. This control involves the Visual control of the candle as well as the analysis of the flame's behaviour during combustion, the shape of the flame, the shape of the wick, the extinction of the wick after the candle is blown.

5. the tests were repeated 4 times during different combustion cycles. For control candles, follow the same protocol, using wax.

EVALUATION TESTS:

Motability test

To assess the toxicity of the oil, a controlled experiment was conducted using sealed containers. Mosquitoes were introduced into these containers, where filter paper treated with the test compound was affixed to the inner surface of the cap. A thin layer of cotton was placed to prevent direct contact between the mosquitoes and the treated paper, and the containers were promptly sealed. The mortality rate of the mosquitoes was monitored after one hour.

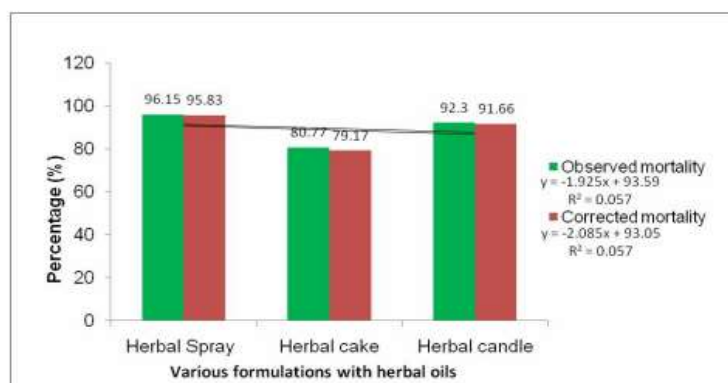


Fig. 6: Comparative efficacy of formulated spray, cake and candle based on mortality.

Insect repellent activity

Aerosol bioassays were conducted following the methodology outlined by Umerie et al. (1998). In this experiment, insects were placed in a Peet-Grundy chamber with a volume of 1 m³, where an aerosol sample, serving as an aerosol repellent, was dispensed. Adult insect mortality was monitored at 5-minute intervals for a duration of 30 minutes. A control group was treated with a 3% Allethrin solution for comparison, while another control set was exposed to deodorized kerosene vapors. The lethal time (LT) was determined by averaging results from three separate replicates. LT values were calculated based on percentage mortality data using probit analysis, as described by Finney (1971). After the exposure period, surviving mosquitoes were returned to a holding tube for a 24-hour recovery phase, during which they had access to a cotton pad soaked in a 10% glucose solution placed on the mesh screen. The mortality rate of the insects was assessed at the conclusion of the recovery period, with percentage mortality adjusted using Abbott's formula (Abbott, 1987).

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

Flammable test

To assess the repellent properties of the prepared candles against mosquitoes and house flies, we evaluated their flammability and burning efficiency, including the duration of the burn and their overall effectiveness as repellents. Flammability tests were performed under controlled laboratory conditions to ensure consistent combustion. Following this, the candles were tested in areas known for mosquito activity, such as chicken shops and residential areas in villages, during evening and nighttime hours. Observations included the burning time of the candles and any associated effects such as irritation or coughing.

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.

Table No. 6 Flammability test for prepared herbal candle

The formulated candles were evaluated in a controlled laboratory setting that mimicked normal room conditions, specifically in an area with a high mosquito population. Each candle, measuring 3 inches, was lit, and key parameters such as flammability, burning efficiency, and repellent effectiveness were monitored. The results indicated that these candles outperformed typical commercial candles in terms of efficacy. The candles exhibited longer burning times and greater periods without mosquito presence, largely due to the integration of potent essential oils like citronella, neem, and patchouli. Previous studies have shown that citronella candles are particularly effective at reducing mosquito bites. Additionally, patchouli oil is known for its strong aroma and effectiveness against insects, even when used in smaller amounts compared to other oils in the formulation.

Stability test

The essential oils were stored at 26 ± 2°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 ± 20°C and 60-80% relative humidity.

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.

Statistical analysis

The values were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) Duncan. The lethal time (LT) 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.

2. ADVANTAGES

1. Herbal candle gives a nice scent.
2. Herbal candle are eco-friend and non-toxic to health.
3. Biodegradable
4. It does not come in direct with skin hence do not produce skin irritation
5. Using of herbal candle reduces environmental impact.
6. It safe for the pregnant women.

3. RESULT

The mosquito repellent activity of neem oil, curcumin and ram Tulsi was studied and proved to be safe and effective.

4. CONCLUSION

Curcumin, Neem and Ram tulsi are considered to be the most efficient herbal mosquito repellants. Today worldwide peoples used synthetic mosquito repellants, which are to harmful and toxic for the respiratory system. there are more harmful than a cigarettes smoke hence we should follow the ancient people, they were used herbal mosquito repellants such as Neem, Curcumin, Ram Tulsi, etc. They used to make smoke by incinerating the leaves and rhizomes of the mentioned herbal plant to kill mosquitos so, I used to make herbal mosquito repellants candles from curcumin neem and ram tulsi which is proved to be most safe and effective mosquito repellants.

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