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DEVELOPMENT AND EVALUATION OF HERBAL MOSQUITO REPELLENT SPRAY

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ABSTRACT

This study presents the development of a natural mosquito repellent spray formulated with lemon and eucalyptus essential oils. Key physicochemical properties—including pH, viscosity, and stability—were evaluated to ensure skin compatibility and formulation consistency. The spray demonstrated a neutral pH of 7.0, smooth flow characteristics (approximately 1.3 seconds through the test nozzle), and remained stable under varying temperature conditions. Utilizing biodegradable, non-toxic components as an alternative to conventional chemical repellents such as DEET, the formulation offers a safer and environmentally friendly approach to mosquito protection.

KEYWORDS: Natural mosquito repellent, Lemon oil, Eucalyptus essential oil, Plant-based formulation, Eco-friendly insect control, DEET.

INTRODUCTION

Being bitten by insects like mosquitoes can lead to diseases such as dengue, malaria, and yellow fever, which can be fatal if not treated on time. All these problems causing insects can be found around Asian countries. To tackle this problem, urban and rural communities with the help of each other need to adopt strong mosquito control strategies. Looking towards finding methods so that reproduction of this sickness with the help of non-toxic insecticides. If we

search in the market we can avail various types of products already available throughout the market but bit has some cons Taking, underage children might experience's problem like irritation like rashes, swelling, eye infections, and other serious complications, due to the potential toxicity these formulations can have on the skin and nervous system. Tackling this rising problem has given us a door for scientists and researchers to make a natural, derived repellent made from bio-based materials rather than chemically synthesized alternatives. If we see there are plants which have natural properties present within them which we can use to for ours good purpose to repellents these harmful insects. In some parts of the world, the use of these chemical pesticides is banned or prohibited due to rising concerns about the development of resistance and natural problems. Shifting to natural ways, we serve the areas.[1] These natural earth repellents provide us with various types of ingredients, such as lemon oil, eyculaptus oil, citronella, etc. It is not limited which only giving us pleasant feelings and aromas, but they also offer significant environmental benefits. Animal sweat that produces and contains lactic acid and carbon dioxide attracts mosquitoes through chemoreceptors located on their antennae. Natural repellents derived from plants have the unique ability to mask these odours, effectively hiding the human scent. Beyond our knowledge, some compounds deter insect feeding by acting in the role of repellents, feeding inhibitors, toxins, or growth regulators. In the way natural repellents carry properties like biodegradability and produce lesser risk to humans and domestic animals, they are considered safer than synthetic alternatives. In consequence, extensive studies are required to explore eco-friendly biological agents as controlling insect pests.[2,3] Diseases, namely malaria, yellow fever, dengue fever, and epidemic polyarthritis, are transmitted by various mosquito species. World Health Organization's assortment details present three million deaths every year. Nonetheless, numerous therapeutic options have been developed to manage mosquito-borne infections such as malaria, but prevention remains the most effective strategy. This knowledge contributed to the innovation of topical applications as well as other preventive strategies against mosquito-borne threats which restrain mosquito bites. Both naturally occurring and synthetic repellents are employed in the following products. About mosquito interactions, countless synthetic repellents, especially chemicals like DEET, get easily absorbed through the skin and have been associated with accidental poisonings, especially among children.[4,5] Additionally, these substances can be very harmful to wildlife. The adverse environmental effect of chemicals, so DDT is doing fine documented, and where as DEET (N, N-Diethyl-meta-toluamide) is suspected retain to have carcinogenic, teratogenic, or mutagenic side effects. Therefore, there is an increasing demand for non-toxic, alternative methods to repel insects. Many studies have figured out natural substances with mosquito-repelling properties. Mosquitoes are responsible for transmitting a wide range of tropical and subtropical diseases that pose serious risks to human health. In the midst, the most commonly found and perilous are malaria, yellow fever, filariasis, schistosomiasis, and the particularly severe dengue hemorrhagic fever, which is spread by *Aedes aegypti*.[8,9] For example, *Wuchereria bancrofti*, which causes filariasis, is spread by the pantropical mosquito *Culex quinquefasciatus*. As a result of transmitting dengue and filarial fever, significant public health concerns in countries such as India have led to increased focus on controlling species like *Aedes aegypti* and *Culex quinquefasciatus*. Mosquito repellents are substances designed to make surfaces unattractive to insects. They can be applied to the skin or other surfaces to deter mosquitoes from landing. Normally, these compositions include subordinate ingredients that contribute cosmetic qualities along with active components that serve as repellents. Various forms are accessible, such as creams, lotions, oils, and most commonly as aerosols; these products sometimes also include devices that emit ultrasonic frequencies, although such devices produce sounds beyond human hearing. Across time, many different materials continued to be used to deter mosquitoes, including tars, clays, oils, plant extracts, and smoke. Most repellents work by interfering with the mosquito's navigational system, which relies on

chemical receptors on its antennae to detect lactic acid, carbon dioxide, and other compounds found in sweat. Lactic acid, naturally released from the skin of warm-blooded animals, activates these receptors. In contrast, evaporative repellents like DEET vaporize when applied. These repellents block the lactic acid receptors, preventing mosquitoes from orienting upwind and causing them to lose track of their target, thereby "masking" the individual from the insect. The subtropical conditions in lieu of Northeast craft perfect breeding environments to diseases like dengue and malaria, transmitted by mosquitoes. Given this, protecting oneself from mosquito bites is of utmost importance. Besides these, while government rules typically suggest regular utilization of insect repellents, with DEET being the most common active ingredient, using DEET for a longer time has shown few side effects, irritation, rashes in tropical areas, headaches, respiratory problems, and even heart attacks. Consequently, there is growing interest in safer, herbal-based repellents. Many local plants contain essential oils that have proven insect-repelling properties. Irrespective of these plants, those in this category are known as weeds, have been used traditionally by certain ethnic groups, and their broader potential remains underutilized.[14,15] In addition to causing allergic reactions such as swelling and stinging, mosquito bites can also transfer bacteria between humans and animals.[18,19] Pathogens responsible for Zika, dengue, and yellow fever are primarily transmitted by the Asian tiger mosquito, *Aedes albopictus* emphasizes that controlling mosquito populations and protecting individuals from bites are key strategies for preventing these diseases. Using repellents can help us achieve our goals. There are two main types of repellents: contact and spatial, each functioning through different mechanisms. According to spatial repellents are highly volatile and disperse in the air within treated areas, with examples including certain synthetic pyrethroids and botanical compounds. Intermittent repellents emit vapours that cause host-seeking mosquitoes to exhibit adverse behaviours and physiological responses, whereas contact repellents like DEET, picaridin, and IR3535 work by directly reducing the signals from olfactory receptor neurons, thus decreasing the mosquito's ability to detect attractive odours. The common method employed in synthetic repellents, combined with DEET, involves direct application to the skin. Found to have numerous health hazards and challenges associated with these chemical repellents. Come to look of essential oils has attracted attention due to lesser toxicity, multiple mechanisms of action, minimal residue, and broad-spectrum activity against mosquitoes. These potent aromatic oils not only give plants their distinctive flavour and fragrance but also provide effective insect-repelling properties. Citronella oil, as an illustration, most broadly used natural repellent before the invention of synthetic options emerged in the 1940s, while lemon, lavender, clove, and eucalyptus oils are also commonly employed. Although synthetic repellents later dominated the market, the demand for safer alternatives has renewed interest in natural essential oils for mosquito repellent applications. Over the years, mosquitoes have increasingly posed significant risks to human health, emphasizing the urgent need for long-lasting solutions to control their populations. Although various methods exist for managing mosquitoes, there is a clear necessity for sustainable and cost-effective strategies. As a result, the researcher aimed to find and develop a natural mosquito repellent that has the following considerations: affordable and environmentally friendly by utilizing locally available plant resources. The primary objective is to create a plant-extract-based pesticide that repels mosquitoes effectively at a low cost, ensuring accessibility, especially in remote areas; to identify the chemical components of the extracts; to assess their mosquito-repelling properties; and ultimately to formulate a potent mosquito repellent.

Fig. 1: Mosquito.

Life cycle of mosquitoes: Learning everything about the different stages of a mosquito's life cycle will provide knowledge to help you prevent mosquito infestation outside and inside your home and choose the right repellents when needed. Every mosquito species goes through four distinct stages during its life cycle.[20,21]

1. **Egg:** The cycle commences when the female mosquito starts to lay her eggs on or near water sources. This could be in rain water, still water, sewage, or even the base of plants where moisture collects. Few mosquito species lay their single eggs, while others lay them in clusters called rafts. These eggs can survive harsh conditions, like dry conditions, for several months, waiting patiently until they come into contact with water. Once any water becomes available, the eggs start to hatch normally within 24 to 48 hours, depending upon the temperature.
2. **Larva:** In conclusion, next, the mosquito enters the larval stage. Larvaers are kind of weak that wriggle inside the water. They are visible easily through the eye in water. Larvae's primary food for their growth are tiny particles in the water, and they grow rapidly, shedding their skin (molting) several times as they develop. This cycle repeats from days to weeks, surviving on favorable temperature and supplies available.
3. **Pupa:** Once fully grown, the larva transforms into a pupa, known as a “tumbler.” Pupae are still aquatic but no longer feed. This has been become the resting stage from which onward they slowly turned into adults. Even in this stage, they remain active and can tumble or roll through the water if disturbed. The pupal stage lasts for days and sometimes a week.
4. **Adult:** The last stage of the mosquito’s life cycle begins when the adult emerges from the pupal case, fully turned and ready. From the pupal case, and rests on the water’s surface as its body and wings harden. Mosquito takes flight in search of its hunt. As we know, only females bite humans or animals. They need blood to develop their eggs and continue the cycle. Once the stomach is fully loaded, they begin to find places to lay their eggs, and the cycle continues.[23,24]

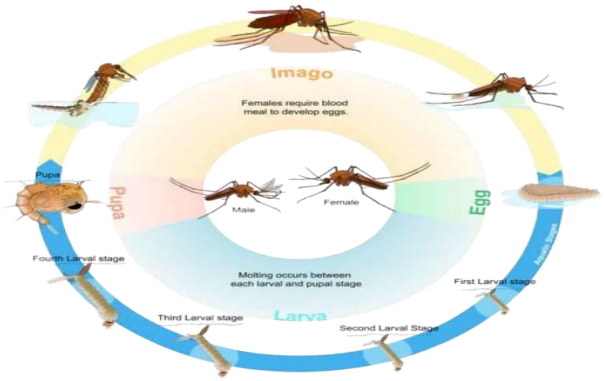

Fig. 2: Life cycle of mosquito.

Mechanism of action of mosquito repellent citrus limon: The mosquito repellent activity of Citrus limon is primarily due to its essential oil limonene, citral, β-Pinene, and γ-Terpinene.

1. Olfactory Disruption

i. Due to their odor receptors, they sense CO_2, lactic acid, and skin odors of the individual.
ii. Limonene and citral interfere with these receptors, making it harder for mosquitoes to locate their host.
iii. This masks the human scent, reducing the mosquito's ability to find and bite.

2. Neurotoxicity in Mosquitoes

i. Limonene acts as a neurotoxin for insects.
ii. It targets the nervous system of mosquitoes and disrupts their neurotransmission interacting through GABA receptors, causing stillness and death.
iii. Inhibiting acetylcholinesterase (AChE), leading to accumulation of acetylcholine, which causes overactivation of nerve signals, resulting in paralysis.[25]

3. Cuticular Penetration

i. The terpenes in lemon oil penetrate the mosquito's cuticle (outer layer).
ii. This causes to dehydration of mosquitoes, killing them.

4. Antifeedant and Deterrent Effect

1. Citral and limonene have an unpleasant smell to repel mosquitoes.
2. The strong citrus scent deters mosquitoes from landing on or near the treated area.

Mechanism of action of eucalyptus as mosquito repellent: The mosquito repellent activity of Eucalyptus is primarily due to its essential oil, which contains 1,8-cineole (eucalyptol), along with α-pinene, limonene, and other terpenes.[16,17]

1. Olfactory Disruption

i. Mosquitoes use odor receptors to detect CO_2 and the skin odors of humans and also animals.
ii. 1,8-Cineole and limonene hide human odors by blocking their receptors, making it difficult to find their target.
iii. This reduces mosquito attraction and prevents bites.

2. Neurotoxicity in Mosquitoes

i. 1,8-Cineole acts on the mosquito's nervous system, disrupting neurotransmission.
ii. Acetylcholinesterase (AChE) is the enzyme that breaks the acetylcholine.
iii. This leads to overstimulation of nerve signals, causing paralysis and death.

3. Cuticular Penetration and Dehydration

i. The terpenes in eucalyptus oil penetrate the mosquito's cuticle (outer layer).
ii. This causes water loss and dehydration, ultimately killing the mosquito.
iii. Limonene and α-pinene intensify the effect by damaging the cuticle barrier.

4. Behavioural Deterrence

i. The strong scent of eucalyptus oil acts as a natural deterrent.

ii. Mosquitoes rarely target the treated portion or areas exposed to eucalyptus oil.[19]

MATERIALS AND METHODS

MATERIALS

All materials used in the formulation of the mosquito repellent spray were sourced from certified suppliers to ensure quality and consistency. Each component was selected for its functional role in enhancing the efficacy, safety, and stability of the final product:

- **Lemon Essential Oil (5.00 mL):** Extracted from fresh lemon peels, this oil is known for its insect-repellent properties and refreshing citrus aroma. It served as the primary active agent in the formulation.
- **Eucalyptus Essential Oil (5.00 mL):** Sourced from eucalyptus leaves, this oil offers complementary insect-repellent activity and a cooling fragrance, working synergistically with lemon oil.[34]
- **Ethanol (95–100%) (50.00 mL):** Used as a solvent and natural preservative, ethanol facilitated the dissolution of essential oils and extended the product's shelf life through its antibacterial properties.
- **Tween 80 (Polysorbate 80) (10.00 mL):** A non-ionic surfactant used to emulsify the hydrophobic essential oils with the aqueous phase, ensuring a stable and homogenous formulation.
- **Distilled Water (30.00 mL):** Utilized to dilute the mixture and adjust viscosity, distilled water was selected to prevent contamination from impurities or minerals.[21]

All materials were handled in a controlled laboratory environment. Measurements were performed using calibrated volumetric flasks and micropipettes. Glassware was sterilized prior to use, and all chemicals were stored according to manufacturer recommendations.

Formulation Procedure

Step 1: Preparation

All glassware (beakers, stirring rods, and storage containers) was sterilized prior to use. Equipment such as micropipettes and stirrers were calibrated to ensure accuracy. Expiration dates and quality certificates of all ingredients were verified.

Step 2: Formation of the Oil Phase

Equal volumes of lemon essential oil (5.00 mL) and eucalyptus essential oil (5.00 mL) were combined in a clean glass beaker and gently stirred using a magnetic stirrer to ensure homogeneity.

Emulsification

Tween 80 (10.00 mL) was added dropwise to the essential oil mixture under continuous stirring. The slow addition was essential to form a stable emulsion. The mixture was visually inspected for homogeneity and absence of phase separation.

Step 3: Incorporation of Ethanol

Using a graduated cylinder, 50.00 mL of 95–100% ethanol was measured and added gradually to the oil-emulsifier mixture while stirring. This facilitated solubilization of the oils and ensured a uniform blend. Care was taken to avoid rapid changes in temperature that could destabilize the mixture.[37]

Step 4: Addition of Distilled Water

Distilled water (30.00 mL) was added slowly, dropwise, to the ethanol–oil mixture under low-speed magnetic stirring. This gradual integration helped maintain emulsion stability and allowed for the formation of a uniform, homogeneous solution.

Step 5: Final Mixing and Packaging

The complete mixture was stirred until no phase separation was observed and a clear, stable solution was achieved. The final formulation was transferred into pre-sterilized spray bottles using a clean funnel, sealed tightly, and stored in a cool, dark environment to preserve the integrity of the essential oils.[40]

METHODS

Step 1: Preparation and Setup

Before beginning the formulation process, all glassware, including beakers, stirring rods, and storage containers, was sterilized to avoid any contamination. The laboratory area was also maintained under controlled environmental conditions to ensure consistency in the preparation process.

Quality Control Check

1. We confirmed the expiration dates and quality certificates for all chemicals.
2. Equipment such as pipettes and stirrers were inspected and calibrated to ensure precise measurements.

Step 2: Blending the Essential Oils with the Emulsifier

The first step in the actual mixing process involved combining the essential oils with the emulsifier. The aim was to create a stable oil phase that would later blend uniformly with the water-based components.[42]

Mixing the Oils

1. In a clean glass beaker, 5.00 mL of lemon essential oil was measured out using a micropipette and carefully poured into the vessel.
2. An equal amount (5.00 mL) of eucalyptus essential oil was then added to the lemon oil.
3. The two oils were mixed gently using magnetic stirring to ensure an even distribution without causing any unnecessary agitation that might oxidize the oils.

Fig. 3: Mixing with magnetic stirrer.

Incorporating Tween 80

1. Tween 80 (10.00 mL) was then introduced gradually into the oil blend.
2. The emulsifier was added dropwise while continuously stirring the mixture at a moderate pace. This gradual addition was crucial for forming a stable emulsion, ensuring that the oil droplets were evenly dispersed in the presence of the surfactant.
3. The mixture was observed visually for signs of homogeneity and to confirm that no immediate phase separation was occurring.[44,45,49]

Step 3: Addition of Ethanol

With the oil-emulsifier blend prepared, the next step was to integrate ethanol into the mixture. Ethanol plays a pivotal role in solubilizing the oils and acting as a preservative.[62,63,64,65,66]

Ethanol Integration

1. Using a calibrated measuring cylinder, 50.00 mL of 95-100% ethanol was measured.
2. The ethanol was slowly poured into the oil-emulsifier mixture while stirring gently. This step ensured that the ethanol was evenly distributed throughout the mixture, thereby facilitating the complete solubilization of the essential oils.
3. The addition was controlled to avoid rapid changes in temperature or concentration gradients that might destabilize the emulsion.[50,51,52,53]

Fig. 4: Addition of Ethanol.

Step 4: Gradual Incorporation of Distilled Water

The next phase involved the careful addition of distilled water to the existing mixture. The water not only adjusts the overall concentration but also helps moderate the formulation's potency, ensuring that the spray is safe for topical application.[67,68,69,70]

Water Addition

1. A total of 30.00 mL of distilled water was measured out with precision.
2. The distilled water was then added dropwise to the ethanol-oil mixture. The slow addition was critical because it allowed for gradual integration, preventing the sudden formation of an unstable emulsion.
3. The mixing was maintained at a constant rate using a magnetic stirrer set at a low speed. This ensured that the water was thoroughly incorporated, forming a uniform solution.[57,58,59,60]

Ensuring Homogeneity

1. The gradual addition of water helped in achieving a smooth consistency with no visible separation between the different components.
2. The process was continued until all the water had been added, and the entire mixture was visually inspected for any signs of separation or inconsistency.
3. At this point, the solution had transitioned from a turbid mixture to a clear, homogeneous liquid, which is essential for both the aesthetic appeal and consistent application of the repellent.

Packaging Process

1. The uniform solution was carefully poured into pre-sterilized spray bottles.

2. Each bottle was filled using a funnel to prevent spillage and maintain cleanliness.

3. The spray bottles were then capped securely to prevent contamination and stored in a cool, dark environment to preserve the efficacy of the essential oils and prevent degradation by light or heat.[71,72,73,74]

RESULTS AND DISCUSSION

After formulation, the mosquito repellent spray was subjected to various tests to evaluate its physicochemical properties, usability, and effectiveness. These assessments ensured the product's stability, safety, and performance in practical conditions. The key evaluation parameters included pH, viscosity, stability under different storage conditions, sprayability and dispersion, and finally, a mosquito repellency test.

1. pH Measurement: The pH of the formulation was measured using a digital pH meter. It is important to maintain a neutral pH to ensure the spray is gentle and safe for human skin. The formulation was found to have a pH value of 7, which is considered ideal for topical applications. A neutral pH ensures that the spray does not cause irritation or disrupt the skin's natural barrier.

Fig. 5: pH testing.

3. **Viscosity:** Viscosity refers to the thickness or flow behavior of the liquid spray. It was measured using a simple time-based method. The time taken for a specific volume of the formulation to pass through a standard orifice was recorded. The observed viscosity was approximately 1.30 seconds, indicating that the spray had a low viscosity and could be easily atomized when dispensed from a spray bottle. This makes it convenient for uniform application over exposed skin areas.

Fig. 6: Viscosity testing.

4. **Stability Studies:** Stability is a crucial parameter to determine whether the formulation remains unchanged under various environmental conditions. The prepared mosquito repellent spray was stored under three different conditions to monitor its physical appearance, separation of phases, and odor over time:

Room Temperature (27°C): The formulation remained stable, with no visible phase separation or degradation of aroma.

Fig.7: Stability testing at room temperature.

Refrigerated Conditions (4°C): The spray maintained its consistency and clarity, indicating good cold stability.

Fig. 8: Low temperature Refrigeration.

Elevated Temperature (40°C): No changes were observed in the formulation, suggesting it can withstand higher temperatures without compromising its integrity.

These stability results confirmed that the spray was robust and could be stored at different temperatures without losing its quality or effectiveness.

Fig. 9: Placement at Elevated temperature.

4. **Sprayability and Dispersion Test:** The effectiveness of the spray mechanism and the distance of dispersion are important for ease of use and proper coverage. The sprayability was assessed by manually spraying the formulation from a standard spray bottle and measuring the distance the liquid could travel while still forming a fine mist.

The spray was tested for its reach and spread

1. The average spray dispersion distance was found to be 15–20 cm, which is ideal for personal mosquito repellent sprays.
2. The mist was uniform, with no clogging observed in the nozzle.
3. This indicates that the formulation was well-suited for practical use, offering easy application and effective skin coverage.

CONCLUSION

This study successfully developed a simple and effective mosquito repellent spray using natural essential oils, primarily eucalyptus and lemon, combined with ethanol and a mild emulsifier. The formulation was gentle on the skin, provided a pleasant fragrance, and delivered a non-sticky, refreshing mist. Stability tests confirmed that the product remained consistent under varying storage conditions, and efficacy trials demonstrated a noticeable reduction in mosquito landings. As an eco-friendly and non-toxic alternative to conventional chemical repellents, this spray offers a practical and sustainable solution for daily mosquito protection.

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