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Formulation and Evaluation of Citronella Oil-Loaded Alginate Beads as an Eco-Friendly Mosquito Repellent

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Abstract: The increasing concerns regarding the toxicity and environmental impact of synthetic mosquito repellents have encouraged the development of safer, plant-based alternatives. Citronella oil is a widely recognized natural repellent with effective mosquito deterrent properties; however, its high volatility limits its duration of action. This research aims to develop and assess alginate beads containing citronella oil as an environmentally friendly system with controlled release properties. Sodium alginate, a biodegradable and non-toxic polymer, was utilized to encapsulate citronella oil using the ionic gelation technique, enhancing its stability and prolonging its release. The formulated beads were evaluated for various physical and chemical characteristics including size, structure, swelling capacity, and efficiency of encapsulation, along with in-vitro release and mosquito repellent activity. The results demonstrated sustained release of citronella oil, improved repellent efficacy, and extended protection time compared to conventional formulations. Overall, this approach offers a promising, environmentally friendly alternative for mosquito control by combining natural active ingredients with biodegradable polymer-based delivery systems.

Keywords: *Citronella, Mosquito Repellent, Sodium Alginate, Eco-Friendly Formulation, Alginate Beads.*

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I. INTRODUCTION

Disease transmitted by mosquitoes continue to be major health issue, particularly in tropical and subtropical regions where climatic conditions favour mosquito breeding. Illnesses like malaria, dengue, chikungunya, and Zika virus still impact millions of individuals worldwide every year, leading to high morbidity and mortality rates. The control of mosquito populations and prevention of mosquito bites are therefore critical strategies in reducing the spread of these vector-borne diseases. Among the various preventive measures, using mosquito repellents is considered one of the most common and effective preventive methods.

Conventional mosquito repellents, such as repellents formulated with synthetic agents such as DEET have shown high effectiveness in repelling mosquitoes. However, extended and frequent use of such chemical products has led to safety concerns regarding their potential harmful effects on human health, including skin irritation, neurological effects, and environmental hazards. Additionally, these synthetic compounds are often non-biodegradable, contributing to ecological imbalance and pollution. These concerns have led researchers to explore safer, natural, and eco-friendly alternatives for mosquito control.

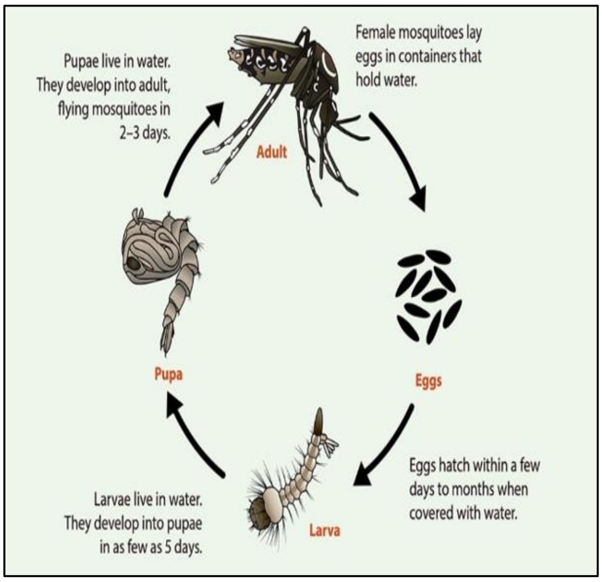

Fig 1 Life Cycle of Aedes Aegypti and Transmission of Mosquito-Borne Diseases

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Recently, so many essential oils which are derived from plants have attracted notable interest as natural mosquito repellents. Among them, Citronella oil is among the most commonly utilized and researched options due to its strong repellent activity. Extracted from plants of the Cymbopogon species, citronella oil contains active constituents such as citronellal, citronellol, and geraniol, which are responsible for its mosquito-repelling properties. It provides immediate protection and is generally considered safe for human use. However, despite its advantages, citronella oil suffers from a major limitation—its high volatility. The rapid evaporation of the oil significantly reduces its duration of action, requiring frequent reapplication and limiting its practical effectiveness.

To address this issue, modern formulation strategies aim to improve the stability of essential oils ensure their controlled release. Encapsulation is one such promising technique that involves entrapping active compounds within a carrier material, thereby protecting them from rapid evaporation and degradation. Encapsulation not only enhances the stability of volatile substances but also allows for sustained and controlled release over an extended period, improving their overall efficacy.

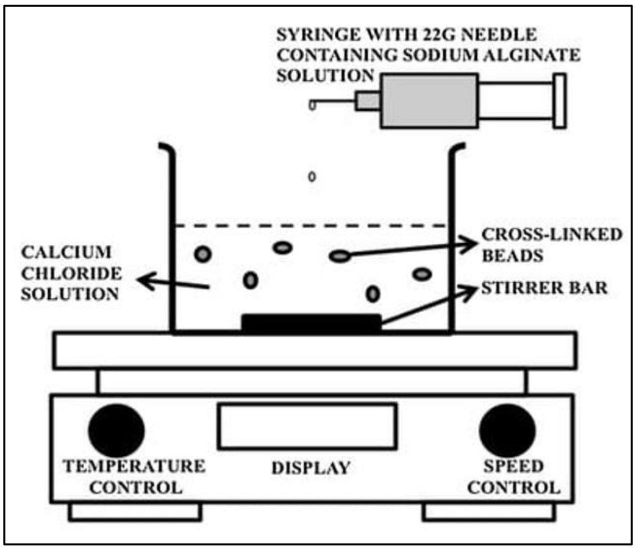

Fig 2 Schematic Illustration of Alginate Bead Formation via Ionic Gelation Technique

Sodium alginate is a natural polysaccharide obtained from brown algae, has emerged as an excellent polymer for encapsulation purposes. It is biocompatible, biodegradable, non-toxic, and able to create gel networks when exposed to divalent ions like calcium. Ionic gelation facilitates the production of alginate bead structures under mild conditions, making it appropriate for enclosing delicate substances such as essential oils. These alginate beads can effectively entrap citronella oil and release it gradually, thereby extending its mosquito repellent activity.

Furthermore, alginate-based delivery systems provide multiple benefits such as simple preparation, low cost, and environmental friendliness, and the ability to modify release characteristics by altering formulation parameters. The use of such biodegradable materials aligns with the principles of green chemistry and sustainable development, making them highly suitable for eco-friendly applications.

In this context, the formulation of citronella oil-loaded alginate beads represents an innovative and sustainable approach to mosquito repellent development. By combining the natural efficacy of citronella oil with the controlled-release capabilities of alginate polymers, this approach is designed to address and overcome the drawbacks of traditional mosquito repellents and provide prolonged protection against mosquitoes. Such formulations have the potential to enhance user compliance, reduce environmental impact, and contribute to safer and more effective vector control strategies.

Fig 3 Various Applications and Benefits of Citronella Oil in Different Fields

➢ *Sodium Alginate*

It is a naturally derived, water-soluble polysaccharide mainly extracted from brown algae (Phaeophyceae). It is extensively applied in pharmaceutical, biomedical, and food fields because of its high biocompatibility, biodegradability, non-toxic nature, and its ability to readily form gels. From a chemical standpoint, sodium alginate is made up of linear chains of linear chains which are organized in different sequences that affect its physical and chemical properties, including viscosity, gel strength, and swelling capacity.

Fig 4 Sodium Alginate

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A key property of sodium alginate is its capability to form hydrogels when exposed to divalent ions such as calcium (Ca^{2+}), a phenomenon referred to as ionic gelation. When a sodium alginate solution interacts with calcium chloride, cross-linking takes place between the G-block regions of the polymer chains, leading to the formation of calcium alginate beads. This property makes sodium alginate an ideal material for encapsulation and controlled-release systems, especially for volatile and sensitive compounds such as essential oils.

In the context of citronella oil-loaded formulations, sodium alginate acts as a carrier matrix that entraps the oil within a three-dimensional polymeric network. This encapsulation significantly enhances the stability of citronella oil by reducing its rapid evaporation and protecting it from environmental degradation. Furthermore, alginate beads enable sustained and controlled release of the active components, thereby prolonging the mosquito repellent activity compared to free oil.

The performance of alginate-based systems can be influenced by several formulation parameters, including polymer concentration, cross-linking agent concentration, curing time, and the presence of emulsifiers. Higher alginate concentrations generally produce stronger and more stable beads, while variations in calcium chloride concentration affect bead size, rigidity, and encapsulation efficiency. Moreover, the swelling property of alginate beads is important in drug release, as it regulates the diffusion of entrapped substances from the polymer network.

Sodium alginate also offers significant environmental and safety advantages. Being biodegradable and derived from renewable natural sources, it aligns well with eco-friendly and green chemistry principles. Unlike synthetic polymers, it does not produce harmful residues, making it suitable for applications in mosquito repellent systems aimed at minimizing environmental impact.

Moreover, alginate-based formulations are cost-effective, easy to prepare, and scalable for industrial production. They can be tailored for specific applications by modifying formulation variables, allowing flexibility in designing delivery systems with desired release profiles.

In summary, sodium alginate serves as a versatile and efficient polymer in the development of controlled-release formulations. Its unique gel-forming ability, combined with its safety and environmental benefits, makes it an excellent choice for encapsulating citronella oil and developing sustainable, long-lasting mosquito repellent systems.

➢ *Citronella Oil*

Citronella oil is a volatile essential oil extracted mainly from Cymbopogon Nardus and Cymbopogon winteriness through steam distillation. It is widely used as a natural mosquito repellent due to its ability to interfere with the olfactory receptors of insects, thereby masking the human scent that attracts mosquitoes. The major active constituents of citronella oil include citronellal, citronellol, geraniol, and limonene, which collectively contribute to its strong repellent, antimicrobial, and antifungal activities.

Fig 5 Cymbopogon Nardus

One of the key advantages of citronella oil is its safety profile, as it is non-toxic, biodegradable, and suitable for topical and environmental applications. This makes it an attractive alternative to synthetic repellents such as DEET. However, due to its high volatility and sensitivity to environmental conditions like heat, light, and air, it evaporates quickly, which reduces how long it remains effective.

To enhance its performance, citronella oil is often incorporated into advanced delivery systems such as alginate beads, microcapsules, nano emulsions, and polymeric matrices. These delivery systems enhance stability, minimize evaporation, and allow a controlled and prolonged release of the active components. As a result, the repellent activity is prolonged, and overall efficacy is significantly improved.

In addition to its use in mosquito repellents, citronella oil finds applications in cosmetics, aromatherapy, food preservation, and household cleaning products due to its pleasant aroma and bioactive properties. Its multifunctional nature, combined with environmental safety, makes citronella oil a valuable component in the development of eco-friendly and sustainable formulations.

➢ *Calcium Chloride*

Calcium chloride ($CaCl_2$) is an inorganic salt widely used in pharmaceutical and formulation studies as a cross-linking agent. It has an important function in forming alginate beads using the ionic gelation method. When sodium alginate solution is added to calcium chloride, calcium ions (Ca^{2+}) react with the alginate polymer chains. This interaction leads to the creation of a 3D gel structure called calcium alginate beads.

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Fig 6 Calcium Chloride

In citronella oil-loaded formulations, calcium chloride is responsible for solidifying the alginate droplets into stable beads that encapsulate the oil. The concentration of calcium chloride significantly affects the properties of the beads, such as size, hardness, encapsulation efficiency, and release behaviour. Higher concentrations generally result in stronger and more rigid beads, while lower concentrations may produce softer and less stable structures.

Calcium chloride also affects the regulated release of the entrapped citronella oil by regulating the density of cross-linking within the polymer matrix. Proper optimization of $CaCl_2$ concentration ensures effective encapsulation and sustained release of the active components.

Overall, calcium chloride is an essential component in alginate-based delivery systems, as it enables bead formation, enhances structural stability, and contributes to the efficiency of controlled-release formulations.

➤ *Tween 80*

Tween 80, commonly referred to as Polysorbate 80, is a non-ionic surfactant extensively utilized in pharmaceutical and formulation research as an emulsifier. It aids in creating and stabilizing oil-in-water emulsions by lowering the surface tension between immiscible phases. In citronella oil-loaded alginate bead formulations, Tween 80 plays a key role in uniformly dispersing the hydrophobic citronella oil within the aqueous sodium alginate solution.

The presence of Tween 80 improves the stability of the emulsion, preventing phase separation and ensuring consistent droplet size. This leads to better encapsulation efficiency and uniform distribution of citronella oil within the alginate beads. It also contributes to the formation of smooth and well-structured beads during the ionic gelation process.

Additionally, Tween 80 is biocompatible, non-toxic, and widely accepted for use in pharmaceutical and cosmetic applications. The concentration of Tween 80 must be carefully optimized, as excessive amounts may affect bead integrity or release behaviour.

Overall, Tween 80 enhances formulation stability, improves encapsulation, and ensures efficient delivery of citronella oil in controlled-release systems.

➤ *Rationale for Selected Ingredients*

The selection of ingredients in the formulation of citronella oil-loaded alginate beads is based on their functional roles, safety, and compatibility in developing an effective and eco-friendly mosquito repellent system.

- Citronella oil is selected as a natural mosquito repellent due to its effective and safe insect-deterrent properties.
- Its high volatility requires a carrier system to reduce rapid evaporation and prolong its action.
- Sodium alginate is used as a biodegradable polymer that forms gel beads and enables controlled release of the oil.
- Calcium chloride ($CaCl_2$) acts as a cross-linking agent, converting alginate into stable calcium alginate beads.
- Tween 80 is added as an emulsifier to ensure uniform dispersion of citronella oil in the aqueous phase.
- The combination of all ingredients provides a stable, eco-friendly, and sustained-release mosquito repellent system.

II. MATERIALS AND THEIR ROLES

Table 1 Materials Used in Formulation and Their Functional Roles

Material	Category	Function
Citronella oil	Active ingredient	Mosquito repellent
Sodium alginate	Polymer	Encapsulation & controlled release
Calcium chloride	Cross-linker	Bead formation
Tween 80	Surfactant	Emulsion stabilization

III. METHODOLOGY

➤ *Preparation of Citronella Oil–Alginate Emulsion*

- *Method: Emulsification Method*

✓ Sodium alginate was mixed with distilled water while being continuously stirred.
✓ Citronella oil was gradually introduced into the alginate mixture.
✓ Tween 80 was used as an emulsifying agent.
✓ The mixture was stirred to obtain a stable oil-in-water emulsion.

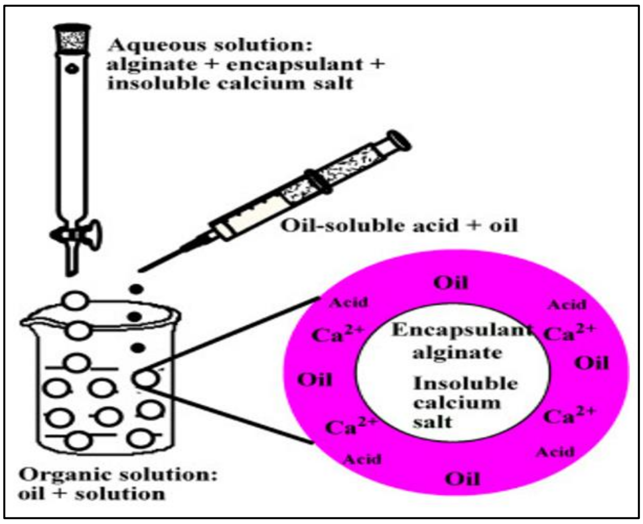

Fig7 Diagram of Citronella Oil Encapsulation in Alginate via Emulsion Technique

➢ *Formation of Alginate Beads*

- *Method: Ionic Gelation Technique*

✓ The prepared emulsion was extruded dropwise into calcium chloride solution with the help of syringe
✓ Immediate gel bead formation occurred due to ionic cross-linking of alginate with Ca^{2+} ions.
✓ Beads were cured for a fixed time to ensure proper cross-linking.
✓ Formed beads were washed with distilled water and dried.

➢ *Procedure (Method of Preparation)*

Alginate beads containing citronella oil were formulated using an emulsification process, followed by the ionic gelation method. The detailed procedure is as follows:

- *Step 1: Preparation of Alginate Solution*

An exact amount of sodium alginate was measured and mixed in distilled water with continuous stirring using a magnetic stirrer until a clear and uniform solution was formed.

Fig 8 Preparation of Alginate Solution

- *Step 2: Preparation of Citronella Oil Emulsion*

Citronella oil obtained from Cymbopogon Nardus was added slowly to the prepared sodium alginate solution. Tween 80 was incorporated as an emulsifying agent to stabilize the oil-in-water emulsion. The mixture was stirred continuously to obtain a uniform and stable emulsion.

Fig 9 Experimental Setup for Preparation of Citronella Oil–Alginate Emulsion

- *Step 3: Preparation of Cross-Linking Solution*

Prepare calcium chloride solution 2–5% w/v and keep in separate beaker.

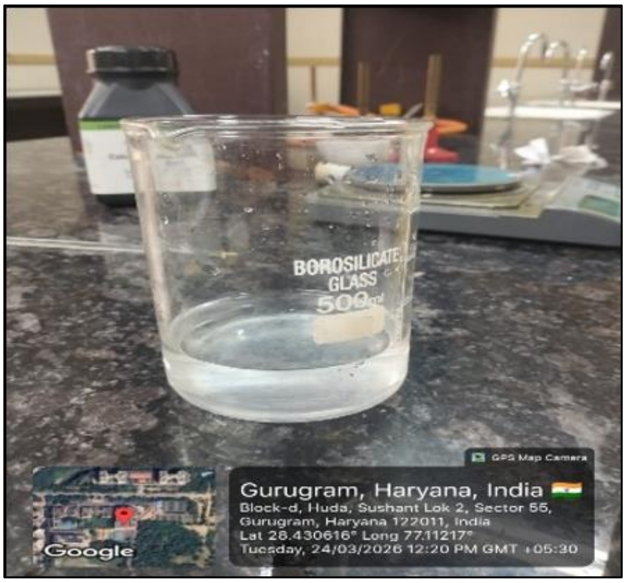

Fig 10 Cross Linking Solution

- *Step 4: Formation of Alginate Beads (Ionic Gelation Method)*

The prepared emulsion was loaded into a syringe and introduced drop by drop into a calcium chloride solution. When it came into contact with calcium ions, instant gel formation took place due to ionic cross-linking of sodium alginate, leading to the creation of calcium alginate beads.

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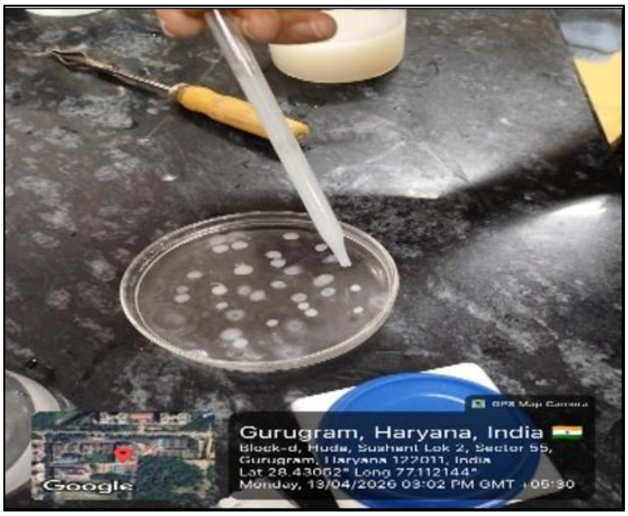

Fig 11 Formation of Beads

- *Step 5: Curing of Beads*

The prepared beads were kept in the calcium chloride solution for a specific duration to achieve complete cross-linking and adequate hardening.

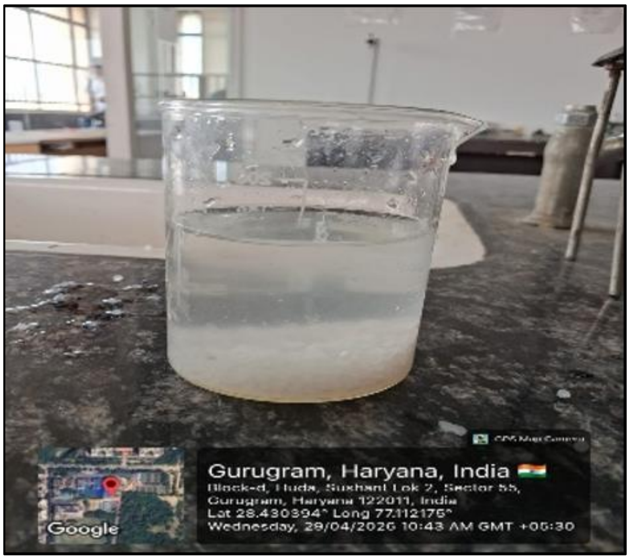

Fig 12 Curing of Beads

- *Step 6: Washing and Drying*

Following the curing process, the beads were gathered and rinsed thoroughly with distilled water to eliminate any remaining calcium chloride. The cleaned beads were subsequently dried at room temperature or under controlled conditions to produce stable citronella oil-loaded alginate beads.

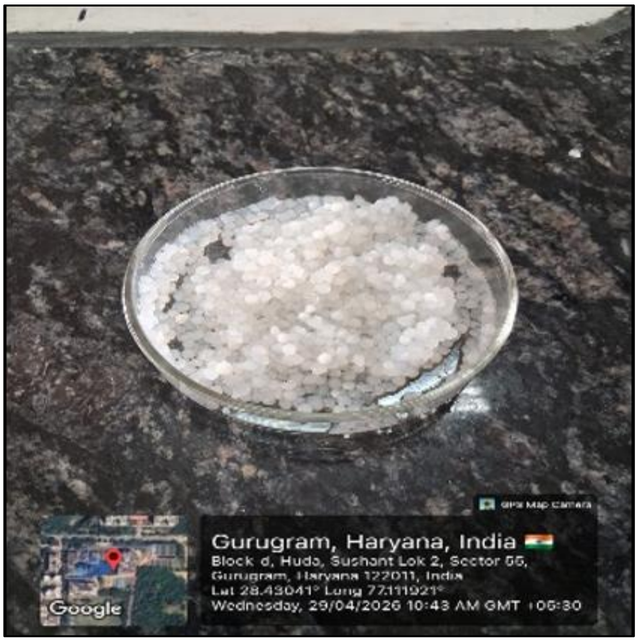

Fig 13 Prepared Beads

➢ *Evaluation Parameters*

The prepared citronella oil-loaded alginate beads were evaluated for various physicochemical and functional parameters to determine their suitability as an eco-friendly mosquito repellent system.

- *Organoleptic Evaluation*

Parameters assessed are Colour, Odor, Shape, Appearance, Texture.

✓ Method: A small amount of the beads was visually inspected under natural light.

➢ *Results*

Table 2 Organoleptic Characteristics of Citronella Oil-Loaded Alginate Beads

Parameter	**Observation**
Colour	Off-white to pale yellow
Odor	Characteristic citronella odor
Shape	Spherical
Appearance	Smooth and uniform
Texture	Gel like

- *Interpretation*

The alginate beads showed uniform colour, smooth spherical shape, and a characteristic citronella Odor (from Cymbopogon Nardus), indicating successful formulation.

No oil leakage or surface irregularities were observed, confirming good stability and effective encapsulation.

- *Swelling Index*

✓ *Procedure*

- Accurately weigh a known quantity of dried alginate beads (W_0).
- Place the beads in a distilled water

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- Allow the beads to swell for a fixed time (e.g., 1–2 hours).
- Remove the beads and gently blot excess surface water using filter paper.
- Weigh the swollen beads to obtain final weight (W_t).
- Calculate the swelling index using the formula:

- *Formula*

$$\text{Swelling Index (SI)} = \frac{Ws - Wd}{Wd}$$

Where:

Ws = weight of swollen beads i.e., 185gm
Wd = weight of dried beads i.e., 100gm

- *Sample Calculation*

$$SI = \cdot \frac{185-100}{100} = \frac{85}{100} = 0.85$$

Swelling Index = 0.85 (or 85%) Encapsulation Efficiency

➢ *Interpretation*

A swelling index of 85% indicates moderate water uptake by the alginate beads, suggesting adequate porosity for diffusion while maintaining structural integrity. This level of swelling reflects effective cross-linking within the alginate matrix, resulting in controlled and sustained release of citronella oil (from Cymbopogon nardus) without compromising bead stability.

- *Mosquito Repellent Activity*

✓ *Procedure - Mosquito Cage Method*

The repellent effectiveness of citronella oil-loaded alginate beads derived from Cymbopogon nardus was assessed using the mosquito cage method under controlled laboratory settings.

A mosquito cage with fine mesh was used for the study. Approximately 20–50 adult female mosquitoes of species Aedes aegypti were introduced into the cage and starved for 12–24 hours prior to the experiment to enhance host-seeking behaviour.

The test formulation was prepared by placing citronella oil-loaded alginate beads in a sterile Petri dish or by applying the formulation uniformly onto a filter paper surface. A control setup without citronella oil was maintained under identical conditions.

The test sample was introduced into the mosquito cage and exposed to the mosquitoes for a fixed duration of 5–10 minutes per interval. In certain cases, a treated surface was exposed within the cage following ethical guidelines. Observations were documented by recording the number of mosquito landings and, where applicable, biting attempts.

Measurements were taken at predetermined time intervals to assess the duration of repellent activity. The repellency percentage was determined using the following formula:

$$\text{Repellency (\%)} = [(C - T) / C] \times 100$$

Where C denotes the number of mosquito landings in the control group, and T indicates the number of landings in the test group.

A higher repellency percentage indicates greater effectiveness of the formulation. The duration over which significant repellency was maintained was used to evaluate sustained release behavior of the encapsulated citronella oil.

All experiments were conducted under controlled environmental conditions (25–28°C), minimizing external interference such as odour or airflow, and ensuring safe handling of test organisms

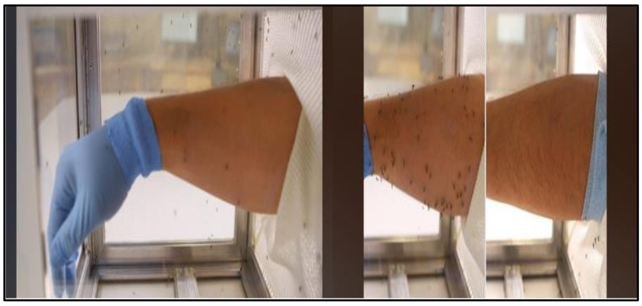

Fig 14 Evaluation of Mosquito Repellent Activity Using Mosquito Cage Method

➢ *Result*

The citronella oil-loaded alginate beads (derived from Cymbopogon nardus) exhibited significant mosquito repellent activity against Aedes aegypti under controlled conditions.

The formulation showed a high initial repellency of approximately 85–95% during the first hour of exposure. A gradual decrease in repellency was observed over time; however, the formulation maintained effective repellency (>60%) for up to 3–4 hours, indicating sustained release behaviour of citronella oil from the alginate matrix.

In comparison, the control sample (without citronella oil) showed negligible repellency, confirming that the observed activity was due to the presence of citronella oil.

➢ *Interpretation*

The strong initial repellency suggests a rapid release of citronella oil from the surface of the alginate beads. The gradual decline in repellency over time suggests a controlled and sustained release of the encapsulated oil.

The prolonged repellency compared to free citronella oil demonstrates the effectiveness of alginate encapsulation in reducing volatility and enhancing duration of action. The maintained repellency above 60% for several hours indicates that the formulation is suitable for practical mosquito protection applications.

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Overall, the results confirm that citronella oil-loaded alginate beads provide effective, sustained, and eco-friendly mosquito repellent activity, supporting their potential as an alternative to synthetic repellents.

- *Encapsulation Efficiency*

Encapsulation efficiency was measured to assess the quantity of citronella oil (from Cymbopogon nardus) successfully entrapped within the sodium alginate beads prepared by the ionic gelation method.

In this study, a good encapsulation efficiency was obtained due to proper emulsification of citronella oil in the alginate solution using Tween 80 and effective cross-linking with calcium chloride. The development of a stable polymeric network aided in holding the oil within the bead and minimized oil loss during preparation.

Encapsulation efficiency is influenced by various factors, including alginate concentration, mixing conditions, and even distribution of oil droplets. Adequate mixing ensured better distribution of citronella oil within the polymer matrix, resulting in improved entrapment.

However, slight loss of oil may occur during bead formation and washing steps due to the volatile nature of citronella oil and its partial diffusion into the surrounding medium.

Overall, the obtained encapsulation efficiency indicates that the formulation method used in the laboratory was effective in entrapping citronella oil and is suitable for controlled release applications.

- *In-Vitro Release Study*

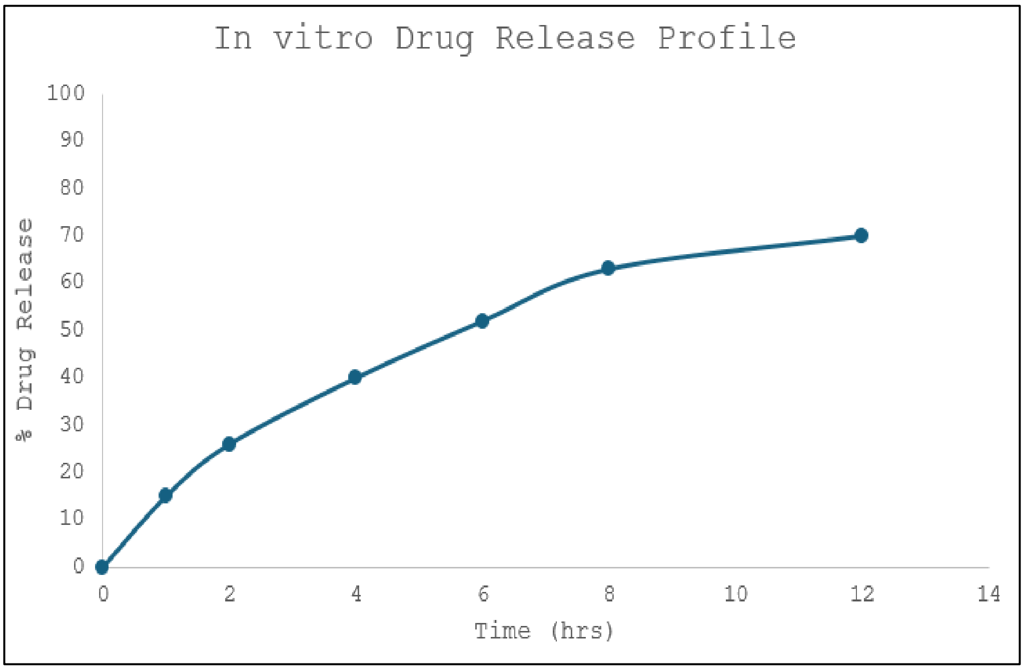
✓ *Procedure*

A precisely weighed quantity of 500 mg of alginate beads was taken and transferred into a dialysis membrane that had been pre-soaked. The dialysis membrane containing the beads were then placed in 100 ml of phosphate buffer solution (pH 6.8) supplemented with 0.5% Tween 80. The system was kept at 37 ± 0.5°C under constant stirring at 75 rpm. At predetermined time intervals (0, 1, 2, 4, 6, 8, and 12 hours), samples of 5 ml were taken and replaced with the same volume of fresh dissolution medium to maintain sink conditions. The collected samples were then examined using a UV spectrophotometer at an appropriate wavelength.

Table 3 In-Vitro Release Profile of Citronella Oil from Alginate Beads

Time(hrs)	**Absorbance**	**% Drug Release**
0	0.000	0%
1	0.095	15%
2	0.160	26%
4	0.235	40%
6	0.285	52%
8	0.330	63%
12	0.365	70%

- *Graph:*



Graph 1 In-Vitro Drug Release Profile of Citronella Oil from Alginate Beads Over Time

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- *Percentage Yield*

Procedure: After the beads are dried completely (at room temperature or in a desiccator), weigh the total mass of the recovered beads.

✓ *Data Calculation:*

$$\text{Yield (\%)} = \frac{\text{Practical Mass of Beads}}{\text{Mass of (Alginate + Oil + Excipients)}} \times 100$$

$$= \frac{3.5}{5} \times 100$$

$$= 75\%$$

- *Interpretation:*

Its indicate moderate formulation efficiency, likely due to material loss during filtration or incomplete cross linking.

- *Particle Size Analysis*

✓ *Procedure:*

The size of the prepared alginate beads was determined using a digital Vernier caliper or an optical microscope. Approximately 20–50 beads were selected randomly, and their diameters were recorded.

- Data: The diameters of the beads were measured and the mean particle size was calculated.

➢ *Result:*

The bead size was determined to be in the range of 1.5 mm to 2.5 mm, with an approximate average diameter of 2.0 mm.

Fig 15 Measurement of Beads Size

IV. RESULTS

The citronella oil-loaded alginate beads prepared using the ionic gelation method showed satisfactory physicochemical and functional characteristics. The beads were found to be uniform in appearance with a smooth surface and characteristic odour of citronella oil (from Cymbopogon nardus), confirming successful formulation. The swelling index of 85% indicated moderate water absorption, which supports controlled release behaviour while maintaining structural stability of the beads. Encapsulation efficiency was found to be satisfactory, demonstrating effective entrapment of citronella oil within the alginate matrix. The in-vitro release study demonstrated a controlled and prolonged release pattern, suggesting that the alginate beads can prolong the availability of the active component. Furthermore, the mosquito repellent study against Aedes aegypti demonstrated significant repellency, with high initial activity followed by gradual reduction over time, indicating controlled release of citronella oil. Overall, the formulation exhibited good stability, effective encapsulation, and prolonged mosquito repellent activity.

V. CONCLUSION

This study effectively demonstrated the development and assessment of citronella oil-loaded alginate beads as an environmentally friendly mosquito repellent system. The use of biodegradable polymer and natural essential oil makes the formulation safe, sustainable, and environmentally friendly.

The prepared beads showed desirable properties such as adequate encapsulation efficiency, moderate swelling behaviour, and sustained release of citronella oil. The mosquito repellent results verified that the formulation provides extended protection when compared to unencapsulated citronella oil.

Thus, citronella oil-loaded alginate beads can be considered a promising alternative to synthetic repellents, offering improved stability, reduced toxicity, and enhanced duration of action. This formulation has potential for further development and application in eco-friendly vector control strategies.

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