# Plant-Based Insect Repellents: A Critical Review of Efficacy, Development, and Testing of Herbal Sprays

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**Abstract**
Mosquito-borne diseases, including malaria, dengue, Zika, and West Nile virus, continue to pose significant global health threats, necessitating the development of effective personal protection measures (1,35). While synthetic repellents such as N,N-diethyl-meta-toluamide (DEET) have served as the gold standard for decades, increasing concerns regarding their neurotoxicity, skin irritation, and environmental persistence have shifted consumer preference toward plant-based alternatives (10,21). This review critically examines the efficacy and development of herbal sprays, focusing on the botanical profiles and repellent properties of guava leaf extract (Psidium guajava), castor oil (Ricinus communis), and marigold extract (Tageteserecta) (3–5). Furthermore, we analyze the role of secondary metabolitesterpenoids, alkaloids, and phenolics—in disrupting insect host-seeking behavior(6,7). The article outlines standardized testing methodologies (WHO/ASTM) and essential safety protocols required to transition these traditional remedies into reliable, commercial-grade biopesticides (1,17). Challenges such as high volatility and the need for advanced formulation techniques like microencapsulation are discussed as the way forward for sustainable insect management (26,31).

## 1. Introduction

The global burden of vector-borne diseases remains a primary concern for public health organizations. According to the World Health Organization, mosquito-borne illnesses account for over 17% of all infectious diseases, causing more than 700,000 deaths annually (1,35). For decades, the primary line of defense has been chemical-based interventions. Synthetic repellents, particularly DEET, picaridin, and IR3535, have provided high efficacy and long-lasting protection (10,19). However, the heavy reliance on these chemicals has led to unintended consequences, including resistance development, environmental bioaccumulation, and adverse human health effects (18,22).

In response to these challenges, there is a growing interest in green chemistry and plant-derived insect repellents (6,40). Traditional plant-based practices are increasingly validated by modern research identifying secondary metabolites responsible for repellent activity (7,39). The development of herbal sprays involves complex botanical chemistry and formulation challenges due to the volatile nature of plant-derived compounds (26,31).

## 2. Botanical Profiles of Key Plants

### 2.1 Guava (*Psidium guajava*)

Psidium guajava leaves have long been used in traditional medicine for antimicrobial, anti-inflammatory, and insecticidal purposes (3,15). Ethnobotanical surveys from tropical regions report the use of crushed or burned guava leaves to repel mosquitoes in domestic environments (6,39). Phytochemical analyses using GC–MS and HPLC have identified terpenoids, flavonoids, and phenolic compounds as the major bioactive constituents (3,7). Volatile components such as 1,8-cineole, α-pinene, and β-caryophyllene interfere with mosquito olfactory receptors, while non-volatile compounds including quercetin and gallic acid contribute antioxidant and insect-deterrent effects (6–8,15).

Laboratory bioassays demonstrate significant larvicidal activity of P. guajava leaf extracts against Aedes aegypti and Culex quinquefasciatus, with low $LC_{50}$ values indicating strong efficacy (3,15,25). Topical formulations reduce mosquito landing rates by more than 60% within two hours of application by masking host-derived attractants such as lactic acid and carbon dioxide (7,12,24). However, rapid evaporation limits protection duration, highlighting the need for improved formulations using fixatives or microencapsulation (26,31).

### 2.2 Castor (*Ricinus communis)*

Castor oil, obtained from the seeds of Ricinus communis, is rich in ricinoleic acid and plays a dual role as both an active repellent component and a fixative in herbal formulations (5,18). Its high viscosity and low volatility slow the evaporation of aromatic compounds, thereby extending the complete protection time of botanical repellents (18,26). Although the castor plant naturally contains ricin, a highly toxic lectin, proper cold-press extraction and refining processes effectively eliminate this toxin, making pharmaceutical-grade castor oil safe for topical application (11).

Beyond its fixative function, R. communis exhibits intrinsic insecticidal activity. The alkaloid ricinine interferes with insect nervous system function, resulting in paralysis and mortality in mosquito larvae and adults (5,25). Castor oil also contributes to physical larval control by forming a hydrophobic surface film that blocks larval spiracles and impairs respiration (25). In topical sprays, it enhances skin adherence, improves spreadability, and reduces irritation caused by concentrated essential oils (21,26). Due to limited volatility, castor oil is most effective when combined with volatile botanical extracts, where it enhances formulation stability and prolongs repellent efficacy (26,31).

**2.3 Marigold (*Tageteserecta*)**

Tageteserecta is widely recognized for its insecticidal and repellent properties, largely due to thiophenes such as α-terthienyl(4,14). These sulfur-containing compounds exhibit strong larvicidal and adulticidal activity, particularly under light exposure, where they generate reactive oxygen species that damage insect tissues (14,33). Marigold essential oil also contains terpenoids including limonene, ocimene, and tagetone, which contribute to its pungent aroma and enhance repellent efficacy (7,20).

Laboratory and field studies report repellency rates of approximately 70–80% against Anopheles stephensi, indicating effective disruption of mosquito host-seeking and landing behavior (4,20). The relatively low volatility of thiophenes allows for prolonged repellent action compared with highly volatile plant oils, making T. erecta suitable for extended-duration topical formulations (26,33). Additionally, marigold extracts demonstrate larvicidal activity in aquatic environments, supporting their role in integrated vector management (14,25). However, the strong odor of marigold oil can reduce user acceptability, and it is therefore commonly blended with milder botanical oils or fixatives to improve sensory properties while maintaining efficacy (7,26,31).

**3. Other Common Herbs in Repellent Development**

In addition to guava, castor, and marigold, several other botanicals are commonly incorporated into herbal repellent formulations to enhance efficacy, broaden the spectrum of activity, and improve user acceptability (6,7). These plants primarily act through volatile secondary metabolites that disrupt mosquito host-seeking and landing behavior (8,39).

**Key Botanical Additives in Herbal Repellents**

- **Citronella (*Cymbopogon* spp.)**
  Citronella oil is widely used due to its high citronellal, geraniol, and citronellol content (7,8). These compounds interfere with mosquito olfactory receptors, producing strong spatial repellency. However, citronella oil exhibits rapid evaporation, resulting in a relatively short protection time of 30–60 minutes unless combined with fixatives or encapsulation systems (7,26).
- **Lemon Eucalyptus (*Corymbiacitriodora*)**
  Lemon eucalyptus oil contains *p*-menthane-3,8-diol (PMD), a plant-derived compound with proven efficacy comparable to low concentrations of DEET (10,21). PMD is the only botanical repellent recommended by the Centers for Disease Control and Prevention for use in disease-endemic areas and provides protection lasting up to 6 hours under field conditions (21).
- **Neem (*Azadirachtaindica*)**
  Neem oil functions primarily as an antifeedant, oviposition deterrent, and insect growth regulator due to the presence of azadirachtin and related limonoids (18,39). While neem-based repellents offer prolonged residual activity, their strong odor often necessitates blending with aromatic oils such as citronella or peppermint to improve user acceptance (7,26).
- **Mint Species (*Mentha* spp.)**
  Peppermint and spearmint oils contain menthol and menthone, which act as potent irritants to mosquito tarsal receptors, preventing successful landing and probing (8,23). These oils also provide a cooling sensation on the skin, enhancing user comfort, though their moderate volatility may limit long-term protection when used alone (23,26).

Collectively, these botanicals are frequently combined in multi-plant formulations to exploit synergistic interactions, improve repellency duration, and reduce the concentration of any single active ingredient **(26,31)**. Such blended approaches form the foundation of modern herbal repellent development and support the advancement of safer, plant-based alternatives to synthetic chemicals **(6,40)**.

## 4. Preparation and Formulation of Herbal Sprays

The transition from raw botanical materials to a consumer-ready herbal spray involves several scientifically controlled stages designed to maximize efficacy, stability, and safety (6,18). Proper formulation is critical, as many plant-derived compounds are volatile, poorly water-soluble, and susceptible to oxidative degradation (26,32).

### 4.1 Extraction

Extraction is the primary step for isolating bioactive compounds from plant materials, and the method selected depends on the chemical nature of the target constituents (6,18).

- **Steam Distillation**
  Steam distillation is widely employed for the extraction of volatile essential oils from aromatic plants such as citronella and marigold (7,14). This method preserves thermally stable terpenoids while minimizing solvent residues.
- **Soxhlet Extraction**
  Soxhlet extraction utilizes organic solvents such as ethanol or hexane to isolate non-volatile alkaloids, flavonoids, and phenolic compounds from guava and castor leaves (3,6). This technique is particularly effective for extracting insecticidal compounds with limited volatility.

- **Cold Pressing**
  Cold pressing is specifically used for castor seeds to obtain high-quality castor oil without thermal degradation of ricinoleic acid (5,18). The resulting oil serves both as an active ingredient and as a formulation base.

### 4.2 Solubilization

Since most essential oils and plant extracts are insoluble in water, solubilization is required to achieve uniform dispersion in spray formulations (17). Emulsifiers such as polysorbate-20 or alcohol-based carriers are commonly used to stabilize oil-in-water systems, ensuring consistent delivery of active ingredients during application (14,17).

### 4.3 Fixation

High volatility is a major limitation of herbal repellents. To address this, fixatives such as vanillin (2–5%) or glycerin are incorporated into formulations (26). These compounds form weak intermolecular interactions with volatile terpenes, slowing evaporation and extending the complete protection time of the spray (26,31).

### 4.4 Preservation

Natural preservatives are added to prevent oxidative degradation of plant oils, which can lead to rancidity, loss of efficacy, and skin irritation (32). Antioxidants such as vitamin E (tocopherol) and rosemary extract are commonly used due to their effectiveness and compatibility with topical products (15,32). Proper preservation also contributes to longer shelf life and improved product safety.

## 5. Standardized Testing Methodologies

For an herbal spray to be considered scientifically credible and suitable for commercialization, it must undergo efficacy and safety evaluation according to internationally recognized protocols, primarily those established by the World Health Organization (WHO) Pesticide Evaluation Scheme and ASTM International (1,17). These standardized methods ensure reproducibility, comparability with synthetic repellents, and regulatory acceptance.

### 5.1 Arm-in-Cage Bioassay (WHO Protocol)

The arm-in-cage bioassay is regarded as the gold standard for evaluating topical mosquito repellents(1,9).

- **Procedure**
  A human volunteer’s forearm is treated with a measured amount of the herbal spray and inserted into a test cage containing 50–100 unfed, pathogen-free female mosquitoes, typically Aedes or Anopheles species (1).
- **Metric**
  The primary outcome is the Complete Protection Time (CPT), defined as the interval between application and the first confirmed mosquito bite. A confirmed failure is usually recorded when one bite is followed by another within a 30-minute observation period (1,9).
- **Controls**
  An untreated arm serves as the negative control, while a standard synthetic repellent such as DEET is used as the positive control to enable direct performance comparison (9,10).

### 5.2 Y-Tube Olfactometer Tests

Y-tube olfactometer assays are employed to assess spatial repellency, which reflects the ability of a repellent to deter insects without physical contact (12).

- **Procedure**
  Insects are released at the base of a Y-shaped glass tube, with one arm containing the herbal odor source and the other containing clean air or a solvent control (12).
- **Metric**
  Repellency is quantified using the Repellency Index (RI), calculated from the proportion of insects that choose the control arm over the treatment arm, indicating avoidance behavior (1,16).

**5.3 Field Trials**

Laboratory bioassays may not fully replicate real-world conditions due to environmental variables such as wind, perspiration, temperature, and humidity (17,37). Field trials therefore play a crucial role in validating laboratory findings.

- **Methodology**
  Human volunteers apply the herbal spray in endemic settings, where mosquito density and species diversity are high, and protection time is recorded under natural exposure conditions (24,37).

**5.4 Larvicidal and Ovicidal Assays**

For plant extracts such as guava and castor that are also intended for larval control, larvicidal and ovicidal assays are conducted (15,25).

- **Metric**
  The $LC_{50}$ (lethal concentration causing 50% mortality) is determined by exposing mosquito larvae or eggs to graded concentrations of the extract in water over a 24–48-hour period (15,25).

These standardized testing methodologies collectively provide a comprehensive assessment of repellent efficacy, persistence, and biological activity, forming the basis for regulatory approval and commercial development of herbal mosquito repellents (1,17).

**6. Safety Protocols and Toxicology**

The designation of a product as "natural" does not automatically equate to safety, and rigorous safety protocols are mandatory for herbal repellents (32).

1. **Acute Dermal Toxicity**

Acute dermal toxicity testing is conducted to ensure that concentrated plant extracts do not cause systemic toxicity when absorbed through the skin (18).

2. **Dermal Irritation and Sensitization**

Many essential oils, such as citronella and oregano oil, are known skin sensitizers. Testing in accordance with OECD Guideline 404 is required to confirm that formulations do not induce erythema, edema, or allergic reactions following dermal exposure(21,32).

3. **Photosensitivity**

Certain plant extracts, particularly citrus-derived oils and thiophenes present in marigold, may cause adverse skin reactions upon exposure to ultraviolet radiation, necessitating photosafetyevaluation(14,33).

4. **Specific Restrictions**

- **Castor Oil:** Castor oil used in topical formulations must be adequately processed to ensure the complete absence of ricin, a highly toxic protein(11).
- **Age Limits:** Health authorities, including the Centers for Disease Control and Prevention and the American Academy of Pediatrics, recommend that certain plant-based repellents such as oil of lemon eucalyptus should not be used on children under three years of age (21).

**5. Environmental Safety**

Environmental risk assessments evaluate the impact of herbal sprays on non-target organisms such as bees, butterflies, and aquatic species. Plant-based repellents generally demonstrate favorable environmental profiles due to rapid biodegradation and reduced bioaccumulation compared with synthetic chemicals(40).

## 7. Challenges and Future Directions

Despite their potential as safer alternatives to synthetic repellents, herbal formulations face several scientific and regulatory challenges (7,22,26).

- **Standardization**

The chemical composition of plant materials, such as guava leaves, varies significantly depending on factors including soil composition, climate, geographic location, and harvest time. This variability complicates the standardization of active ingredient concentrations and reproducibility of repellent efficacy (22,31).

- **Longevity**

Most plant-based repellents provide effective protection for only 2–3 hours due to the high volatility of essential oils, necessitating frequent reapplication and limiting user compliance(7,26).

- **Regulatory Hurdles**

Regulatory requirements for herbal repellents vary widely across regions. In some countries, these products are classified as "minimum-risk pesticides," while in others they are subject to the same costly and time-consuming registration processes as synthetic chemicals (11,18).

**Future Directions: Microencapsulation**

Recent advances in formulation science focus on microencapsulation of herbal oils within polymer-based carriers such as chitosan or lipid nanoparticles (26). These microcapsules adhere to the skin surface and enable controlled, slow release of volatile compounds over 8–12 hours, potentially matching or exceeding the protection duration of DEET-based formulations (26,31). This approach represents a promising strategy for overcoming the primary limitations of herbal mosquito repellents.

## 8. Conclusion

Plant-based insect repellents derived from Psidium guajava, Ricinus communis, and Tageteserecta offer effective and biodegradable alternatives to synthetic chemicals. Although herbal formulations often require more frequent application, advances in formulation science, particularly the use of fixatives and encapsulation technologies, are steadily improving protection duration.

The successful use of these natural repellents depends on strict adherence to standardized efficacy testing and safety evaluation protocols established by international authorities . With continued research and validation, plant-based repellents can contribute meaningfully to sustainable mosquito control and public health protection.

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