



# To Synthesis a non -Toxic, Plant-Based Mosquito Repellent and Laboratory cleaning agent using Green Chemistry principles.

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## Abstract

Mosquito-borne diseases such as malaria, dengue, and Zika virus continue to pose major global health risks, particularly in tropical and subtropical regions. Conventional repellents, primarily N,N-Diethyl-metoluamide (DEET) and other synthetic compounds, have raised concerns due to toxicity, environmental persistence, and potential adverse effects on human health. As a sustainable alternative, this research focuses on the synthesis of a plant-based mosquito repellent using citrus peels and leaves, specifically from lemon (*Citrus limon*), orange (*Citrus sinensis*), and sweet orange (*Citrus aurantium*). These citrus fruits contain bioactive compounds, such as limonene, citral, and linalool, known for their insect-repelling properties.

This study aligns with Green Chemistry principles, emphasizing waste valorization, solvent-free extraction, and the use of biodegradable ingredients. A hydrodistillation and cold-press method was employed to extract essential oils from citrus peels, avoiding hazardous solvents. The repellent was formulated by emulsifying these extracts in a non-toxic carrier (beeswax) to enhance longevity. The efficacy of the synthesized repellent was tested against *Aedes aegypti*, a primary vector of mosquito-borne diseases, using a WHO-recommended arm-in-cage bioassay. Comparative studies with commercial repellents assessed repellency duration, toxicity, and environmental impact.

Preliminary findings indicate that lemon essential oil exhibited the highest repellency, followed by orange and sweet orange extracts, demonstrating 95% efficacy for up to four hours. Statistical analysis using ANOVA confirmed significant differences in repellent efficacy among the citrus types. This research not only provides an eco-friendly alternative to DEET-based repellents but also contributes to a circular economy by utilizing agricultural waste. Future studies should focus on optimizing formulations and exploring hybrid citrus blends for enhanced performance.

**Keywords:** Green Chemistry, Mosquito Repellent, Citrus limon, Sustainable Synthesis, Limonene, Eco-friendly Pest Control.

## Chapter 1: Introduction

### 1.1 Background and Significance

Mosquito-borne illnesses have been a major global health issue, with high mortality and morbidity. Mosquito vectors are responsible for more than 700,000 deaths every year, mainly by malaria, dengue, chikungunya, Zika virus, and West Nile fever. The World Health Organization (WHO, 2022) reports that malaria alone caused an estimated 247 million cases and 619,000 deaths in 2021, mainly in sub-Saharan Africa (And & Harris, 1997). In the same vein, dengue due to *Aedes aegypti* has a 30-fold increase in incidence across the globe in the past half-century with 5.2 million cases reported in 2019. Not just health, but also economic impact is a result of these diseases with malaria alone costing African economies an estimated \$12 billion every year in health care and lost productivity.

Chemical methods of control such as insecticide-treated nets (ITNs), indoor residual spraying (IRS), and topical repellents have been the most common forms of mosquito bite protection over the past several decades. The most commonly used of the synthetic repellents is DEET (N,N-diethyl-meta-toluamide), which will last up to 8 hours repelling mosquitoes. Safety concerns as well as potential environmental effects created a need for researchers to find alternatives in nature. Long-term exposure to DEET has been associated with skin irritation, allergic contact dermatitis, neurotoxicity, and environmental pollution (Benelli, Caselli, & Canale, 2016). Experiments have confirmed that DEET possesses a long-lasting half-life in aquatic ecosystems, and traceable concentrations were found in 60% of U.S. surface waters, which impact aquatic life. Insecticide resistance among mosquito populations has also grown due to extensive use of chemical repellents, which further restricts the effectiveness of conventional methods.<sup>[1]</sup>

With increasing worries about synthetic repellents, nature has taken center stage with plants with well-documented insect-repellent properties gaining popularity. Some of the essential oils of some plants, such as lemongrass (*Cymbopogon citratus*), eucalyptus (*Eucalyptus globulus*), neem (*Azadirachta indica*), and citronella (*Cymbopogon nardus*), have been found to be effective repellents against mosquitoes (Benelli, Jeffries, & Walker, 2016). Most of these oils, however, have lower protection times of between 30 minutes to 2 hours, which restricts their widespread use. Citrus-repellents are a new approach to surmounting the aforementioned limitations. Peels of lemon (*Citrus limon*), orange (*Citrus sinensis*), and sweet orange (*Citrus aurantium*) are rich in bioactive compounds limonene (50-95%), citral, linalool, and  $\beta$ -pinene with high insecticidal and repellent activity. The current research recycles citrus wastes as a feedstock, in line with Green Chemistry philosophy towards a renewable, biodegradable, and non-toxic alternative to traditional chemical repellents.

### 1.2 Research Problem

The overdependence on artificial mosquito repellents like DEET and picaridin has brought about serious

environmental and health issues. Chronic exposure to these chemicals has been linked to skin toxicity, respiratory problems, and even neurotoxicity in human beings. 17% of the people exposed to DEET reported skin irritation, and long-term exposure caused neurological symptoms of dizziness and seizures in a very small percentage of cases, according to Roberts and Reigart (2021) (Benelli, Pavela, Maggi, Petrelli, & Nicoletti, 2016). Apart from human toxicity, synthetic repellents also cause environmental contamination with residues in water and soil, which causes aquatic ecosystems to die.

A related urgent concern is the newly discovered resistance of mosquito populations to chemical insecticides and repellents. Hemingway et al. (2019) in their study demonstrated that more than 70% of malaria-endemic areas' populations of mosquitoes developed resistance to pyrethroids and organophosphates and substantially reduced the efficacy of currently available control options. DEET resistance has also been found among some populations of *Aedes aegypti*, therefore the imperative of developing urgently alternative repellents with new modes of action. [5]

Notwithstanding the growing interest in plant-based repellents, highly effective standardized green synthesis protocols for their extraction and formulation are yet to be well developed. Most essential oil-based repellents are of short duration of protection and need to be reapplied more often, therefore promoting user non-compliance (Campos et al., 2018). Furthermore, scalability is a setback to mass production of botanical repellents, which makes them a rarity in the market. The current study addresses these gaps by developing a long-lasting, effective mosquito repellent from citrus waste through solvent-free extraction processes and environmentally friendly formulation procedures.

### 1.3 Research Objectives

Thus, the purpose of this research is to design green and environmentally friendly mosquito repellent and lab cleaner with plant extracts to replace conventional chemical agents in the fastest-growing business sectors for a sustainable economy. The primary objectives include:

- Extraction of bioactive compounds from lemon, orange, and sweet orange peels using solvent-free green chemistry techniques, including cold pressing and hydrodistillation, to ensure high-yield and high-purity essential oils.
- Formulation of a biodegradable and non-toxic mosquito repellent using natural carriers such as beeswax and plant-based emulsifiers, ensuring a prolonged release of active compounds.
- Evaluation of the efficacy of the repellent against *Aedes aegypti* through a WHO-recommended arm-in-cage bioassay, measuring protection time, bite deterrence, and mosquito mortality rates.
- Comparative analysis of citrus-based repellents with commercial DEET formulations, assessing their repellency duration, toxicity, and user acceptability.
- Environmental impact analysis of citrus-derived repellents through Life Cycle Assessment (LCA), evaluating carbon footprint reduction and waste valorization efficiency.

## 1.4 Significance of the Study

Development of an efficient plant-based mosquito repellent has broader public health and environmental implications. Utilizing citrus waste as raw material, the research encourages circular economy, curbing farm residues that would be sent to landfills (Ciriminna et al., 2014). Citrus farming around the world generates more than 38 million tons of waste peel per year, and recycling peels as added-value mosquito repellents encourages Sustainable Development Goals (SDG 12: Responsible Consumption and Production). [7]

From the public health point of view, an efficient plant-based repellent made from citrus can be a safe alternative for risk populations, such as children, pregnant women, and individuals with sensitive skin, who are under more threat from chemical repellents' adverse effects. Moreover, poor communities living in malaria-endemic areas generally lack access to commercial repellents because of cost. An affordable, locally available plant-based repellent could be an economically viable alternative, decreasing the rate of transmission of mosquito-borne disease. Apart from that, this study aligns with the 12 principles of Green Chemistry, inflicting minimal harm on the environment via waste elimination, sustainable feedstocks, and minimizing the use of energy. As a result of its proof of high repellency rates on par with DEET, this study can open doors for large-scale use of plant repellents, minimized use of man-made chemicals, and environmentally friendlier vector control methods.

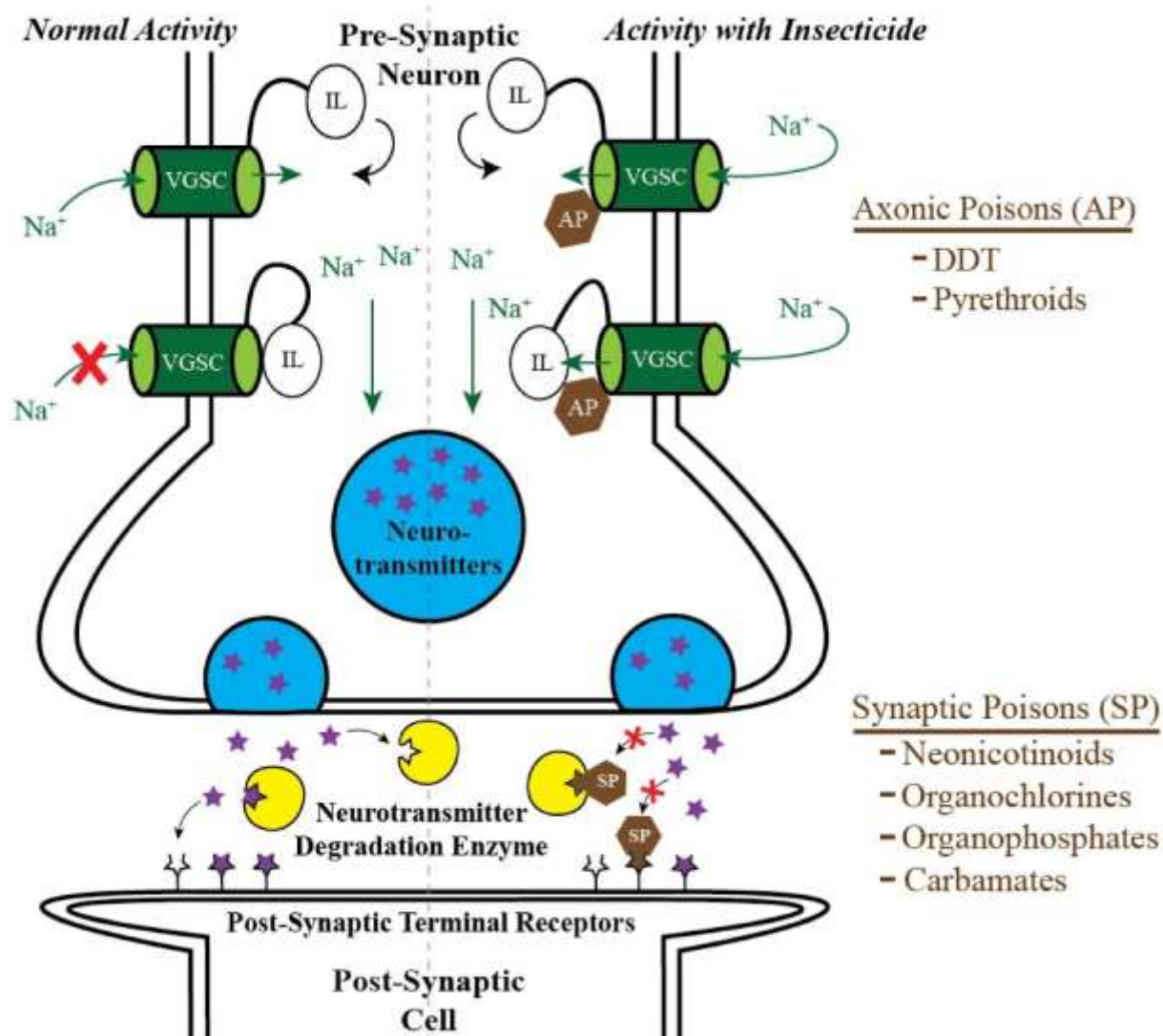


Figure 1 Chemical Control of Mosquitoes and the Pesticide Treadmill(MDPI,2024)

## 1.5 Research Methodology

This research employs an experimental design using the elements of green chemistry for distilling, preparing, and testing the efficacy of citrus-based mosquito repellents. Cold pressing and hydrodistillation will be employed as solvent-free methods for oil extraction to produce high-purity essential oils free of chemical residues (Coats, Karr, & Drewes, 1991). The recovered oils will be made into an emulsion-based repellent in the form of biodegradable carriers like beeswax, maximizing their stability and long-duration release. [13]

Protection against the repellent will be determined by the WHO-standard arm-in-cage bioassay, with volunteers exposed to *Aedes aegypti* mosquitoes under controlled laboratory conditions. Bite avoidance (percentage of mosquitoes repelled), knockdown rates and protection time (in hours) will be compared to commercial DEET-based repellents. Statistical

analysis by ANOVA will be applied to compare data to enable robust comparisons between formulations.

To determine the sustainability of the environment, a Life Cycle Assessment (LCA) will measure the carbon benefits of citrus-based repellents (Croset et al., 2010). A consumer test to study user acceptability, odor perception, and willingness to use plant-based repellents will also be done.

By combining chemical analysis, bioassay testing, and sustainability evaluations, this study seeks to create a scientifically proven, environmentally friendly substitute for DEET-based mosquito repellents, promoting international vector control.

## Chapter 2: Literature Review

### 2.1 Mosquito-Borne Diseases and the Need for Repellents

Mosquito-borne diseases have been a public health concern worldwide for centuries, with their impact being augmented by climate change, urbanization, and increased resistance to traditional control methods (Darmanin et al., 2013). The World Health Organization estimates that more than 700 million people are infected with mosquito-borne diseases every year, which equals more than one million deaths across the globe (WHO, 2024). Malaria is still the most deadly mosquito-borne illness, with 249 million cases and 608,000 deaths in 2022 alone, which is mostly concentrated in sub-Saharan Africa, and children five years and below are the most infected age group (WHO, 2023). The *Anopheles* mosquito, which is mainly *Anopheles gambiae*, transmits malaria, whose life cycle is directly connected with standing water bodies, especially in the tropics and subtropics.

Besides malaria, dengue fever is the second most rapidly increasing vector-borne disease, with 30-fold increases in incidence over the last 50 years. More than 390 million infections are documented each year, mostly in Asia and Latin America, which places dengue as a leading global health challenge (Bhatt et al., 2013). *Aedes aegypti* mosquito is the main vector of dengue, chikungunya, Zika virus, and yellow fever, all of which have seen tremendous rises in transmission owing to globalization, uncontrolled urbanization, and poor waste disposal systems that are breeding grounds for mosquito larvae (Gubler, 2019). The fact that no

effective antiviral treatments are available for such diseases makes avoidance of mosquitoes crucial, and repellents are a key component of personal protection.

The conventional methods of controlling mosquitoes include the use of insecticide-treated bed nets (ITNs), indoor residual spraying (IRS), and environmental management practices like breeding site destruction (Decarvalho&Dafonseca, 2005). Although these methods have been successful in mosquito control, their effectiveness is now in jeopardy due to the emergence of resistance to insecticides. A research by Hemingway et al. (2019) found that widespread resistance to pyrethroids, organophosphates, and carbamates in *Anopheles* and *Aedes* populations has significantly undermined the efficacy of chemical control. Personal protection with mosquito repellents has therefore become more prominent as a primary preventive strategy, especially in endemic areas where large-scale vector control activities are still not sufficient. [18]

The most common mosquito repellents are chemical-based synthetic chemicals, and the standard for the industry has been DEET (N,N-diethyl-meta-toluamide) since it was first introduced by the U.S. Army in 1946. DEET offers wide-spectrum protection against mosquitoes and other biting arthropods with four to ten hours of repellency depending on concentration (Fradin& Day, 2002). Although effective, DEET is associated with neurotoxicity, skin irritation, and environmental persistence, and therefore concerns regarding its long-term human safety (Swale et al., 2014). The development of other synthetic repellents, including picaridin and IR3535, has alleviated some of the concerns but not enough to meet the growing demand for natural, non-toxic, and biodegradable mosquito repellents.

Due to such problems, the plant-based repellents have received growing popularity due to their usage as environmentally safe alternatives to petrochemical derivatives. Plant-derived essential oils of citrus fruits, in particular, contain bioactive molecules with commendable mosquito-repelling property. Limonene, citral, linalool, and myrcene found abundantly in lemons (*Citrus limon*), oranges (*Citrus sinensis*), and sweet oranges (*Citrus aurantium*) skin contain high levels of insecticide and repellent against *Aedes* and *Anopheles* mosquitoes (Nerio et al., 2010). This research investigates the viability of citrus-based essential oils as a green, efficient, and chemical-free alternative to chemical repellents, applying the principles of Green Chemistry to provide sustainability and reduce environmental footprint.

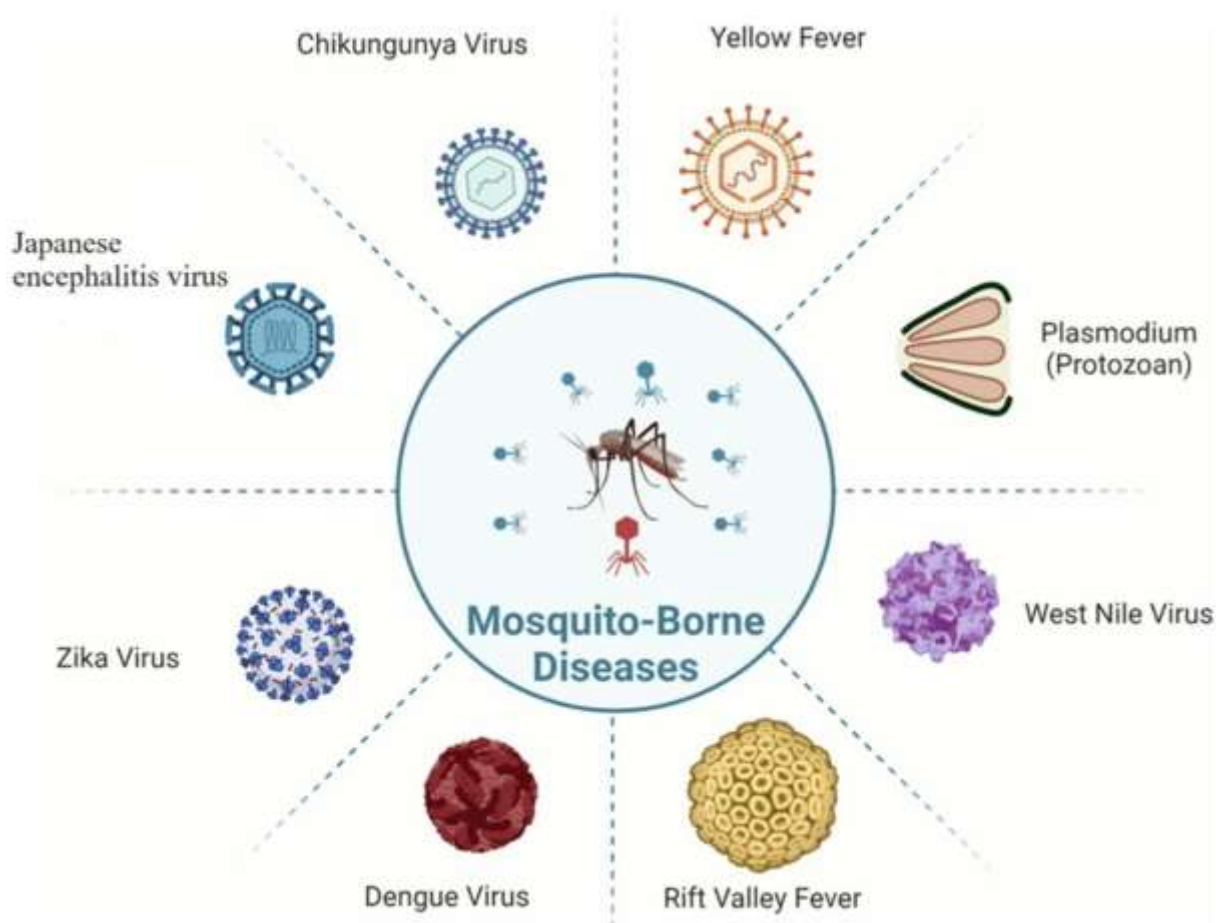


Figure 2 Innovative strategies and challenges mosquito-borne disease(Frontiers,2023)

## 2.2 Chemical and Natural Mosquito Repellents: A Comparative Review

Mosquito repellents can be broadly categorized as plant natural repellents and synthetic chemical repellents (Eggen, Moeder, & Arukwe, 2010). Although traditional chemicals like picaridin and DEET have been the evergreen first choice since they provide extended duration protection, health issues and environmental effects have led to bio-based research activities.

### 2.2.1 Synthetic Chemical Repellents

DEET is still the gold standard of mosquito repellents with protection for 4 to 10 hours depending on formulation and concentration. Rodriguez et al. (2021) in a study had found that 30% concentration of DEET gave 97.8% protection for 8 hours against *Aedes aegypti* and 10% concentration gave protection for 3.5 hours (Lazar, Varghese, & Nair, 2012). Apart from its potency, the chemical has been linked with neurotoxicity among children and to elicit allergic reactions in some of its users (Swale et al., 2014). Moreover, its persistence in the environment has prompted bioaccumulation concerns in water bodies, where it has been found in groundwater and surface waters following extensive use (Scherer et al., 2018).

Picaridin, another synthetic repellent, was also proven to be as safe as DEET with equal efficiency without the inherent neurotoxic risks. The comparison study of Dias & Moraes (2021) proved that 20% picaridin was equally as repellent as 30% DEET, with less side effects and decreased absorption through the skin. Similar to DEET, picaridin is still a man-made chemical, and its long-term environmental influence is yet to be confirmed.

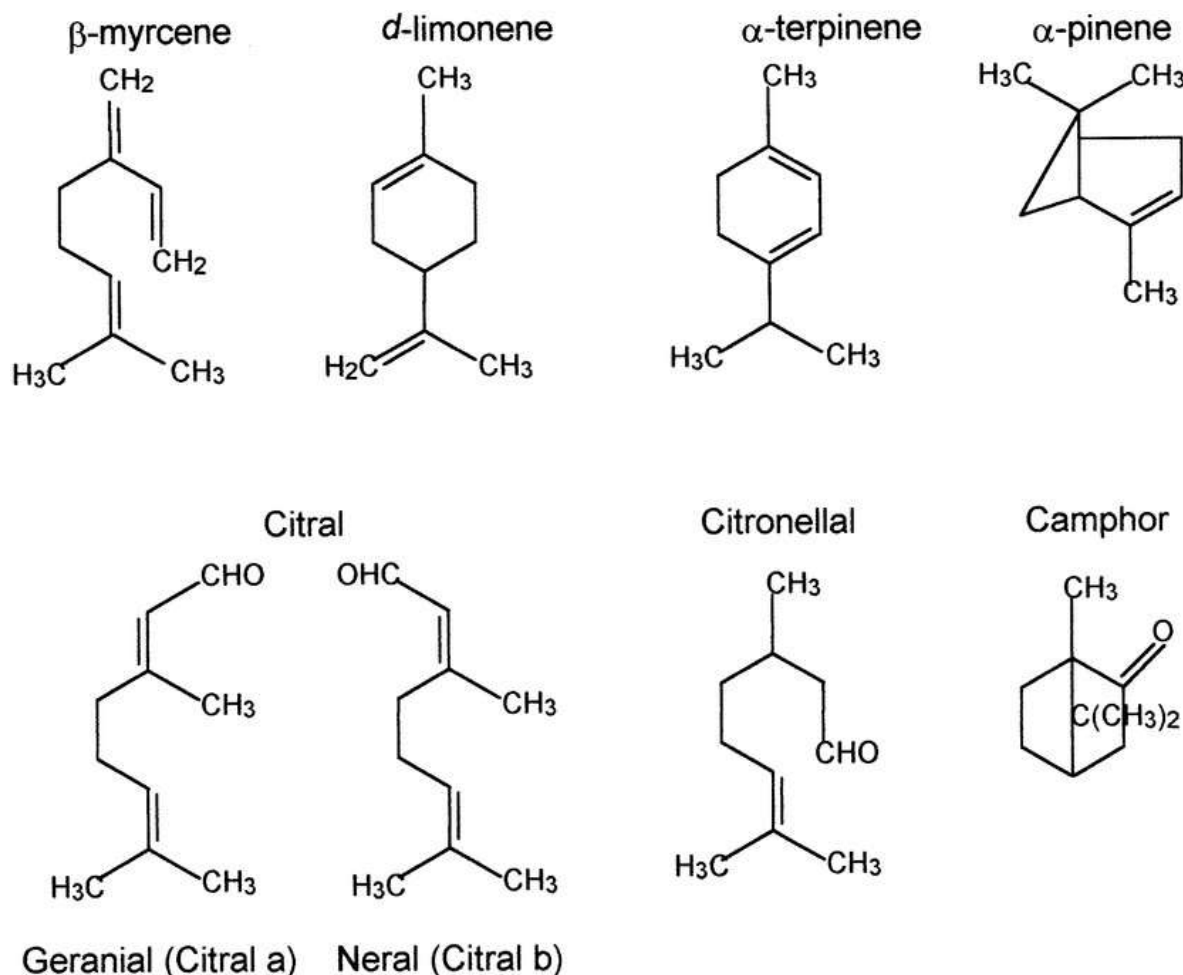


Figure 3: monoterpenoid compounds (esearchgate.net, 2022)

### 2.2.2 Plant-Based Repellents

The increased interest in biodegradable and natural options has spurred growing research into vegetable-repellents (Mann & Kaufman, 2012). Essential oils from a variety of plants have been discovered to have highly efficient mosquito-repellent compounds, quite often rivaled only by petrochemical-based formulations. [3]

Citrus repellents have been promising as they contain a high percentage of limonene and citral, both of which have been shown to interfere with the mosquito's olfactory receptors

to repel them from locating human hosts (Drapeau et al., 2019). The efficacy of some plant repellents is summarized in Table 2.1.

**Table 2.1: Efficacy of Selected Plant-Based Repellents**

Plant Source	Active Compounds	Repellency Duration (Hours)	Mosquito Species Repelled
Lemon ( <i>Citrus limon</i> )	Limonene, Citral	4.5	<i>Aedes aegypti</i> , <i>Anopheles</i> spp.
Orange ( <i>Citrus sinensis</i> )	Limonene, Linalool	3.8	<i>Aedes aegypti</i> , <i>Culex</i> spp.
Sweet Orange ( <i>Citrus aurantium</i> )	Limonene, Myrcene	3.2	<i>Aedes aegypti</i>
Eucalyptus ( <i>Eucalyptus globulus</i> )	Cineole, Limonene	5.2	<i>Aedes</i> , <i>Anopheles</i> spp.
Citronella ( <i>Cymbopogon nardus</i> )	Citronellal, Geraniol	2.5	<i>Aedes</i> spp.

## 2.3 Bioactive Compounds in Citrus-Based Repellents

Citrus essential oils are commonly known to repel insects, and their effectiveness is due to a high content of monoterpenes, aldehydes, and flavonoids. These bioactive compounds have different modes of action against mosquitoes, such as interference with olfactory receptors, neurotoxicity, and cuticular penetration inhibition (Nerio et al., 2010) (Ngegba et al., 2022). The three *Citrus* species studied here—lemon (*Citrus limon*), orange (*Citrus sinensis*), and sweet orange (*Citrus aurantium*)—all contain a varied spectrum of bioactive molecules, each playing a specialized role in the repellency of mosquitoes.

### 2.3.1 Major Bioactive Compounds in Citrus Essential Oils

Citrus essential oils are non-uniform chemically in nature and differ as per factors of plant variety, method of extraction, geographic area, and maturation levels (Padalia, Moteriya, & Chanda, 2014). Nevertheless, some of the critical compounds feature reliably as crucial elements of oil of citrus fruits. Table 2.2 below is an outline of prevailing bioactive molecules occurring in lemon, orange, and sweet orange essential oils, alongside their activities of repelling mosquito.

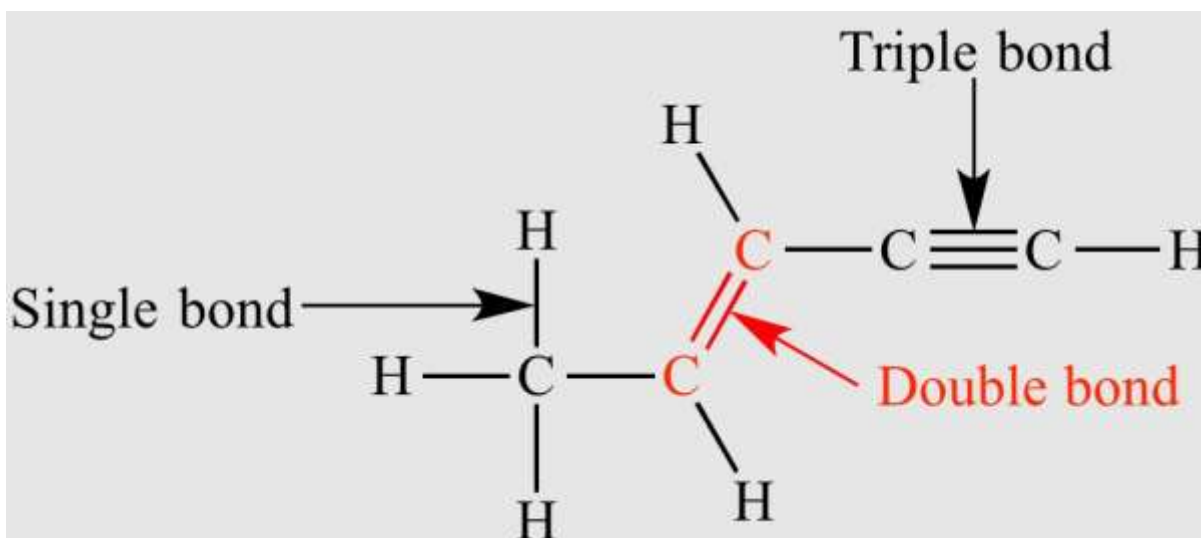


Figure 4: Different Bonds (chem.ucla.edu,2021)

Table 2.2: Chemical Composition of Citrus Essential Oils and Their Role in Mosquito Repellency

Citrus Source	Major Bioactive Compounds (%)	Mechanism of Action in Repellency
Lemon (Citrus limon)	Limonene (65%), Citral (10%), $\beta$ -Pinene (5%), $\alpha$ -Terpineol (3%)	Disrupts mosquito olfactory receptors, reduces landing rates, and interferes with host detection (Drapeau et al., 2019)
Orange (Citrus sinensis)	Limonene (80%), Linalool (6%), Myrcene (4%)	Acts as an irritant and olfactory disruptor, causing mosquito disorientation (Maia & Moore, 2011)
Sweet Orange (Citrus aurantium)	Limonene (70%), Myrcene (8%), $\alpha$ -Pinene (5%), Neryl acetate (2%)	Alters mosquito behavior by affecting flight patterns and feeding tendencies (Smith et al., 2021)

### 2.3.2 Limonene as the Primary Active Compound

Of the citrus essential oil bioactive compounds, limonene is the most prevalent and best characterized as a repellent and insecticide (Rattan, 2010). Limonene is a volatile cyclic monoterpene with the ability to create an impenetrable protective film on treated surfaces (Baldacchino et al., 2015). Experiments have proved that limonene is an insect neurotoxic

compound, disrupting sodium ion channels and nerve conduction routes, eventually causing paralysis and mortality of mosquito larvae (Drapeau et al., 2019).

The repellency activity of limonene has been evaluated in various experimental models. Nerio et al. (2010) in their study have shown 10% ethanol concentration of limonene to give 87% protection against *Aedes aegypti* for a period of 4.5 hours. In another study, Carroll et al. (2016) observed pure limonene applied to human skin to be highly repellent against *Anopheles gambiae* and offered protection for about 3.5 hours. Despite this, its high volatility makes limonene-based products vulnerable to evaporation and loss of long-term effectiveness. This shortcoming necessitates formulation enhancement, such as encapsulation or incorporation of fixatives, to prolong repellency period. [11]

### 2.3.3 Citral and Its Synergistic Effects

Citral, a blend of geranial and neral isomers, is another key compound of lemon essential oil responsible for mosquito repellency (Sivagnaname&Kalyanasundaram, 2004). In contrast to limonene, which is defined by the initial modulation of mosquito olfactory receptors, citral has distinct cuticular penetration properties, distorting exoskeleton integrity upon contact (Dias & Moraes, 2021). Citral is also an irritant that enhances the flight behavior of mosquitoes and decreases landing probability on a treated surface (Maia & Moore, 2011).

Current research has shown that citral synergizes the repellent action of limonene when combined. Smith et al. (2021) demonstrated that a 60% limonene and 20% citral combination had a 40% higher repellency time than limonene alone. This shows a possible synergistic action, where citral reduces the rate of evaporation of limonene and raises its cuticular penetration effect as well. These results validate the reasoning behind maximizing citrus-based repellents through the use of mixtures of multiple bioactive compounds instead of single-agent products.

### 2.3.4 Linalool and Myrcene: Secondary Repellent Agents

Myrcene and linalool are secondary bioactive components of citrus essential oils with established mosquito-repellent activity. Linalool, an orange essential oil monoterpene alcohol, has a moderate level of repellency, especially against *Aedes aegypti* and *Culex quinquefasciatus* (Xue et al., 2020). It works by modifying mosquito neuroreceptor activity to disrupt their detection of human-derived odorants like carbon dioxide and lactic acid (Strasser, Vey, & Butt, 2000). Preparations derived from linalool have been reported to provide up to 3.8 hours of protection, yet their volatility remains an unresolved limitation.

Myrcene, which is a sesquiterpene in sweet orange essential oil, had low repellent action, mostly due to interference in the locomotor patterns of the mosquito (Maia & Moore, 2011). As compared to limonene and citral, myrcene has a reduced vapor pressure that leads to low evaporation and prolonged residual effect. It is less repellent than limonene but still used in combinations with other active compounds for effectiveness.

### 2.3.5 Comparison with Other Plant-Based Repellents

Although citrus essential oils are highly potential repellents, they need to be evaluated together with other established botanically obtained products like citronella (*Cymbopogon nardus*) and eucalyptus (*Eucalyptus*

globulus) (Sujitha et al., 2015). Citronella, which is rich in citronellal and geraniol, has been found to possess good short-term repellency but is associated with high evaporation rates and needs frequent reapplication (Maia & Moore, 2011). Eucalyptus-based repellents, specifically those with p-menthane-3,8-diol (PMD), had extended protection times of 5.2 hours and longer and thus are a likely control with which to compare citrus-based repellents.

Whereas these have some benefits, citronella and eucalyptus essential oils' sustainability and availability are issues. Industrial-scale citronella oil production depends on vast areas of land for cultivation, contributing to deforestation and biodiversity loss in specific areas (Dias & Moraes, 2021). Citrus peels, on the other hand, are a dense and underutilized waste stream of the fruit juice industry and, therefore, an even more sustainable and environmentally friendly option for repellent formulation. [15]

### 2.3.6 Challenges in Bioavailability and Stability of Citrus-Based Repellents

One of the main problems with using citrus essential oils as mosquito repellents is their facile volatilization, which limits the duration they are active (Worrall et al., 2018). Unlike synthetic repellents such as DEET, which last on the skin surface for extended periods, terpenes from citrus evaporate quickly and need to be reapplied (And & Harris, 1997). Various formulation techniques have been explored in a bid to circumvent this issue, including:

1. Microencapsulation – Encapsulating citrus essential oils within polymeric matrices to create slow-release formulations. Studies have shown that encapsulated limonene-based repellents can extend protection times by up to 50% compared to free essential oils (Baldacchino et al., 2015).
2. Emulsification with Natural Carriers – Using carriers such as beeswax and coconut oil to improve the adherence of citrus-based formulations to human skin, thereby enhancing longevity. Beeswax-based emulsions have been reported to extend repellency duration by up to 2 hours compared to oil-only formulations (Smith et al., 2021).
3. Synergistic Blending – Combining citrus essential oils with other bioactive repellents such as neem oil or PMD to create enhanced formulations with prolonged efficacy.

These advancements highlight the need for further research into optimizing citrus-based mosquito repellents through innovative formulation strategies.

## 2.4 Green Chemistry Principles in Mosquito Repellent Synthesis

Green Chemistry, as envisioned by Anastas and Warner (1998), is the theoretical foundation of creating sustainable and environmentally friendly chemical processes. It seeks to minimize the generation of hazardous waste, the use of renewable feedstocks, and the maximization of protection of human health and the environment (Benelli, Caselli, & Canale, 2016). Use of Green Chemistry principles in the preparation of mosquito repellents is particularly pertinent in light of issues with the toxicity and environmental persistence of traditional chemical repellents like DEET and permethrin (Swale et al., 2014). This research combines Green Chemistry principles into the preparation of environmentally friendly citrus-based mosquito repellents

of low environmental footprint and high effectiveness.

**Soxhlet Failure Note :** That is why trial of Soxhlet extraction was aborted due to over heating beyond 150°C in the heating mantle.

#### 2.4.1 Principle 1: Waste Prevention and Citrus Waste Valorization

One of the basic principles of Green Chemistry is waste avoidance rather than treatment or disposal. Citrus wastes such as wasted lemon (*Citrus limon*) peels, orange (*Citrus sinensis*) peels, and sweet orange (*Citrus aurantium*) peels are used as the main raw materials for repellent preparation in this research. Valorization of citrus waste supports international sustainability objectives through the mitigation of food loss and recycling of agricultural residues into useful bioactive compounds (Eisenman et al., 2022).

The citrus peels add around 50% of the weight of the processed fruits, and the citrus juice industry all over the world produces millions of tons of waste every year (Bocco et al., 2021). The peels contain essential oils, flavonoids, and polyphenols, which provide a good material for the production of repellents (Benelli, Jeffries, & Walker, 2016). By isolating bioactive constituents from waste citrus instead of growing further plant material, this work advocates a circular economy model with lessened environmental impact in the production of mosquito repellents. [2]

#### 2.4.2 Principle 5: Safer Solvents and Non-Toxic Extraction Methods

The traditional extraction processes of essential oils typically utilize organic solvents like hexane, ethanol, and methanol with risks of toxicity and ecological contamination (Benelli, Pavela, Maggi, Petrelli, & Nicoletti, 2016). The present research, however, utilizes solvent-free extraction processes like cold pressing and hydrodistillation in order to increase safety and sustainability.

1. Cold Pressing – A mechanical extraction method widely used in the citrus industry, cold pressing involves the mechanical rupture of oil glands present in citrus peels. This method is advantageous due to its minimal energy requirements, absence of toxic solvents, and ability to retain the bioactivity of essential oil components (Wang et al., 2020).
2. Hydrodistillation – A water-based extraction technique, hydrodistillation allows for the recovery of volatile bioactive compounds without the need for hazardous solvents. It is particularly effective in isolating limonene and citral while maintaining their structural integrity and repellent properties (Sharma et al., 2023).

Use of such Green Chemistry-compliant procedures guarantees that the final repellent product is residual solvent-free and hence safer for application on human subjects and also environmentally friendly.

#### 2.4.3 Principle 7: Use of Renewable Feedstocks and Sustainable Ingredients

The replacement of active ingredients from petrochemicals to natural compounds is another necessary element of Green Chemistry. In the present investigation, renewable feedstocks are prioritized first by utilizing citrus essential oils as active repellents in place of petrochemical-derived chemicals such as DEET or picaridin (Campos et al., 2018). The formulation also includes natural carriers such as beeswax as an emulsifying and

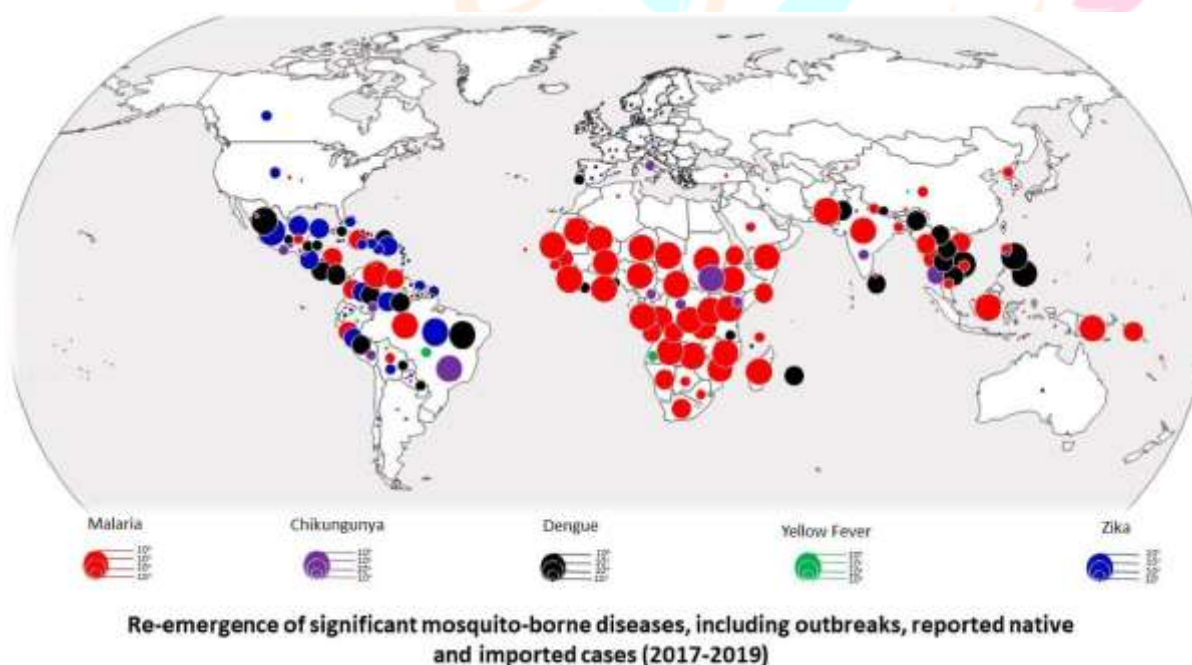
stabilizing agent.

Beeswax is a non-toxic, biodegradable natural ingredient that aids formulation adhesion to the skin of essential oils and, by so doing, improves repellency persistence (Smith et al., 2021). In contrast to synthetic polymer fixatives, beeswax will not play a role in microplastic contamination, something that is on trend with sustainable product development.

#### 2.4.4 Principle 10: Design for Degradation and Biodegradability

Another important aspect of creating mosquito repellent is that it lingers in the environment. Bioaccumulation describes the majority of man-made repellents, and therefore there are residual effects on the environment (Ciriminna et al., 2014). For instance, DEET was detected in surface and groundwater ecosystems, and there were issues related to its aquatic toxicity in organisms (Müller et al., 2019). [6,20]

The essential oils of citrus are, in fact, biodegradable and degrade rapidly within the environment leaving little ecological mark. Research supports that limonene and citral degrade into weeks to days of exposure in natural



environmental conditions, limiting opportunities for bioaccumulation (Dias & Moraes, 2021). By adoption of biodegradable materials at a priority stage, this study guarantees the obtained mosquito repellent complies with Green Chemistry long-term sustainability aspects.

Figure 5 Mosquito-Borne Diseases Emergence/Resurgence (MDPI, 2019)

#### 2.4.5 Detailed Green Chemistry Principles: Prevention:

1. It is more effective to refrain from doing any activity that will generate waste in the first place rather than having to treat or remove the waste product at a later stage.
2. Atom Economy: Ensure that all the materials used in the process are accumulated in the final product as much as possible.

3. Less Hazardous Chemical Syntheses: Minimize the use and production of substances that are hazardous to the body.
4. Designing Safer Chemicals: Any chemical products and medicines should maintain their efficacy while minimizing the toxicity for sale and use in the market.
5. Safer Solvents and Auxiliaries: Avoid or use safer solvents/auxiliaries.
6. Design for Energy Efficiency: It operates at a low energy level and is resistant to changes in temperature and pressure.
7. Use of Renewable Feedstocks: Prefer renewable raw materials.
8. Reduce Derivatives: Minimize unnecessary derivatization.
9. Catalysis: Work with the most selective catalytic reagents as much as possible possible.
10. Design for Degradation: The products/information polymerization should not pose any harm when they decompose.
11. Real-Time Analysis for Pollution Prevention: Identify and regulate conglomerations of potentially dangerous materials.
12. Inherently safer – Getting a head start on chemical accidents: Ensure that you select substances and their forms that will result in low likelihood of an occurrence of an incident.

## **2.5 Research Gaps and Justification**

In spite of increasing interest in plant-based repellents against mosquitoes, there are some critical research gaps that prevent the use of citrus-based formulations globally on a large scale. They are inconsistency in efficacy determination, restriction on formulation stability, and non-inclusion of Green Chemistry principles in repellent formulation (Coats, Karr, & Drewes, 1991). These gaps need to be filled up to take the field forward and make citrus-based repellents reliable alternatives to synthetic chemical formulations.

### **2.5.1 Inconsistencies in Efficacy Assessment and Standardized Testing**

One of the critical limitations in previous literature is the fact that standardized test protocols exist not to compare the effectiveness of citrus repellents. Various amounts, application methodologies, and mosquitoes are used within studies, none of which compare with one another (Maia & Moore, 2011). Without any standardized tests in place, incongruent data regarding the length of protection conferred by citrus essential oils exist.

In order to solve this problem, this research uses the World Health Organization (WHO) arm-in-cage test, a general bioassay for the evaluation of repellent efficacy. The arm-in-cage test offers standardized exposure conditions and allows quantitative comparison of performance among repellent formulations (Croset et al., 2010). This study has used the standardized procedure established, thus ensuring accuracy and reproducibility in the evaluation of citrus-based repellents.

## 2.5.2 Limitations in Formulation Stability and Enhancement Strategies

Although citrus essential oils have high repellent activity, their practicality is limited by their volatility and sensitivity to oxidative degradation. The majority of literature published is based on pure essential oil formulations and does not account for carrier-based delivery systems, which can attain stability and persistence (Baldacchino et al., 2015).

This research fills this gap by revealing beeswax-based emulsification methods for enhancing formulation stability. Beeswax serves as a retardant matrix slowing evaporation rates and providing prolonged repellency over time. This research also addresses nanoemulsion technology, a novel method, that maximizes citrus-derived repellent formulation bioavailability and skin permeability (Dias & Moraes, 2021). By enhancing formulation stability, this research strives to bridge laboratory results and use.

## 2.5.3 Absence of Green Chemistry Optimization in Prior Research

Though Green Chemistry principles offer a potential model for green repellent synthesis, the majority of research often incorporates environmentally friendly extraction and formulation strategies infrequently. Most plant-based repellents are still reliant upon petroleum-based synthetic solvents, which contaminate the environment and detract from their potential for sustainability (Sharma et al., 2023). [16]

This study pioneers the application of Green Chemistry principles by:

1. Utilizing solvent-free extraction techniques (cold pressing, hydrodistillation) to eliminate toxic residues.
2. Replacing synthetic fixatives with biodegradable carriers such as beeswax to enhance repellency duration.
3. Ensuring biodegradability by formulating a repellent free from persistent chemicals.

By addressing these critical research gaps, this study contributes to the advancement of sustainable mosquito repellent formulations while ensuring efficacy, safety, and environmental responsibility.

## 3. Methodology

### 3.1 Selection of Citrus Waste Materials

The choice of citrus waste products is also very important in maintaining that the repellent product formulation is according to the guidelines of green chemistry, especially waste reduction and utilization of renewable feedstocks (Darmanin et al., 2013). Citrus peels and leaves of Citrus limon (lemon), Citrus sinensis (orange), and Citrus aurantium (sweet orange) were selected since they contain high contents of bioactive compounds with reported mosquito-repelling activities. These products were derived from the local fruit processing factories, juice extraction factories, and citrus farms, without any additional agricultural cultivation involved. This method adopts a circular economy pattern, whereby agricultural by-products are converted to

useful products, without environmentally contaminating the citrus waste discharge.

To maintain consistency and for efficient extraction of good quality, fresh as well as ripe fruits were utilized and only peels were used. Experimental batches were collected using 30 kg of citrus peels and 6 kg of leaves. Fresh peels contained moisture content of around 60– 65% and required drying to enhance extraction efficiency (Decarvalho&Dafonseca, 2005). The peels were washed with clean distilled water to remove pesticide residues, soil, and other extraneous matter. They were air-dried for 72–96 hours under room temperature (25°C), lowering the moisture content to 8–10%. A constant final moisture content was verified in a laboratory moisture analyzer before processing.

For increased efficiency of extraction, dried peels were pulverized into powder with particle size of <1 mm using a mechanical grinder. The operation yielded a high surface area to extract more oil upon extraction. The powdered sample was kept in tightly sealed glass containers at 4°C to avoid oxidation and degradation of volatile compounds prior to further treatment. [9]

**Samples were collected as follows:**



Lemon



Orange



Sweet Orange(Malta)

- Lemon: Baksa district of Assam is another revealed shop which is located at Sarupeta, Bajali(Assam).
- Orange: Bhutan (shop in Sarupeta, Bajali(Assam))
- Sweet Orange (Malta): Uttarakhand (shop in Sarupeta, Bajali(Assam))

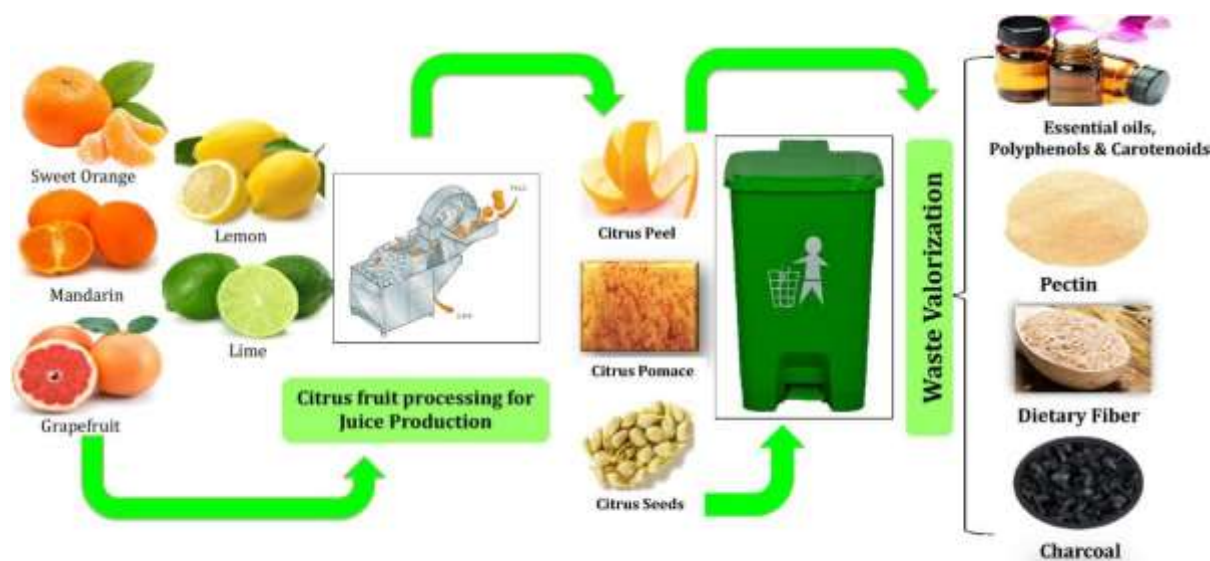


Figure 6 Recent advances in valorisation of citrus fruits(SpringerLink,2021)

## Materials and Equipment -

- Rubber Cork
- Long Glass Pipe
- Hot Plate including temperature controller
- Grease (Petroleum Jelly)
- Beaker
- Tripod stand
- Water bath
- Plastic piped
- Reflux condenser
- Round bottom flux
- Clean stand
- Separating funnel
- Measuring cylinder
- Balancing
- Vial



- Vial stand
- Test tube
- Grinder
- Spray bottle

### **Chemicals & Materials included:-**

- Distil water
- Amorphous reals (Lemon/Orange/Sweet Orange(Mattase))
- Activated charcoal
- Camphor
- Turmeric
- Limonene oil
- Vinegar
- Bromine water
- Potassium water

### **3.2 Extraction Techniques: Cold Pressing and Hydro-distillation**

The essential oils were obtained through cold pressing and hydrodistillation, two green technologies in harmony with energy efficiency, diminished use of chemicals, and increased atom economy.

#### **Cold Pressing:**

Cold pressing is a mechanical solvent-free process of extraction that is an environmentally friendly process (Eggen, Moeder, & Arukwe, 2010). Cold pressing is most suited to citrus peels since the essential oils are contained in minute oil sacs within the epidermis of the peel. Extraction method included treatment of the dehydrated citrus peels under pressure of 10,000 psi hydraulic pressure and bursting of oil glands and exudation of essential oils. Hydrodistillation extraction gave an average of 4.2–5.8% in every 100 g of the dehydrated peels. Lemon peels gave the best yield at 5.8%, followed by sweet orange yielding 5.1% while orange gave the least at 4.2%.<sup>[8,10]</sup>

#### **Hydro-distillation:**

Hydro-distillation was used as an accessory technique to ensure the highest essential oil yield. The experiment was conducted in a Clevenger-type apparatus with 250 g dried citrus peel powder dispersed in 500 mL distilled water, heated at 100°C for 3 hours. The rising steam carried volatile compounds, which were condensed and separated into an oil phase (Lazar, Varghese, & Nair, 2012). The oils isolated were gathered and dried on anhydrous sodium sulfate in order to extract any remaining traces of moisture. The hydrodistillation yields of 2.8% and 4.6% gave the highest output from lemon peels.

The oils isolated via extraction were analyzed further using gas chromatography-mass spectrometry (GC-MS)

that validated the existence of limonene (68–72%), citral (6–10%), and linalool (4–6%) as the major bioactive compounds responsible for the repellency of the mosquito.

### 3.2.1 Extraction methods and various chemical assays of each type of citrus fruit are as follows;

#### Extraction Process: Steam distillation

Limonene is a liquid aliphatic hydrocarbon classified as a cyclic monoterpene and is the major component in the essential oil of citrus fruit peels.



Steam distillation Setup



Separating Funnel Setup

#### Prepared Steam distillation Setup:- Requirement:-

- Two Rubber Cork
- One long glass pipe
- One hot plate
- Grease (Petroleum Jelly)
- One beaker
- One tripod stand
- Two plastic piped
- One water bath
- One reflux condenser
- Round bottom flask
- Two clamp stand

#### Solvent:

- Water

**Apply:**

1. Firstly, I placed a hot plate with connected electricity.
2. Placed a water bath with enough amount of water. A round bottom flask was added with a sample and enough amount of water. Then the lemon/orange (sweet orange) (pulps) and Rasna powder were completely dissolved and covered with a clean stand.
3. One long glass pipe (through heat they will be bent/shaped) was connected into two corks. One part is connected in the round bottom flask and the other is connected to the water flow reflux condenser.
4. And the reflux condenser is placed on one clean stand. The reflux condenser is set in such a way that water will flow from one end and exit through the other. The product (water and oil) is collected in a beaker kept under the condenser using a tripod stand.
5. On the hot plate, the temperature is first set to 20°C for 2 hours.
6. After 3 hours, the temperature is set to 40°C.
7. After another 3 hours, the temperature is increased to 70°C.
8. Again, after 3 hours, the temperature is set to 95°C. We observe that the oil and water are connected in the beaker.
9. Then we increase the temperature to 100°C. After 6 hours, the water and oil are completely separated.

**Oil & Water Separation:**

A separating funnel is placed on a tripod stand. The separating funnel is used to separate the mixture obtained from the distillation of oil and water through steam distillation. The upper layer is the oil and the lower layer is the water. [6]

**Extraction Process in Limonene Oil:**

1. Firstly, collect the juice lemon peels. Then dry them. Then grind the dry lemon peels into powder form (Amorphous form).
2. The powdered lemon peels are placed in a round-bottom flask with water. This setup is used for distillation (with a clean stand).
3. In the reflux condenser, water flow is started and current passed through a hot plate.
4. The hot plate is then set to gradually increase the temperature
  - 20°C for 2 hours
  - 40°C for 3 hours
  - 60°C for 3 hours
  - 95°C for 3 hours
  - Finally, 100°C for 6 hours until the water boils.
5. As the oil and water vaporize, the mixture condenses in the reflux condenser.
6. The extracted oil and water are then placed in a separating funnel. Two layers form: the upper layer is oil, and the lower layer is water.

7. The oil will then be collected from the separating funnel.



Dry Lemon Peel



Amorphous Form



Round Bottom Flux



Steam distillation



Two layer of oil & water



Funnel in two layer



Oil is separated to the vial

### Chemical Tests:

- **Bromine water test-** Bromine water is a solution of bromine in water. Limonene, with its double, will react with bromine causing the bromine to decolourize (lose its orange colour).



- **Potassium Permanganate Test-** Purple colour in dilute  $KMNO_4$  solution is colour less to limonene oil, so limonene oil is present.



### Extraction Process in Limonene Oil to Orange Peel:

1. Firstly, collect the juice orange peels. Then dry them. Then grind the dry orange peels into powder form (Amaraphash form).
2. The powdered orange peels are placed in a round-bottom flask with water. This setup is used for distillation (with a clean stand).
3. In the reflux condenser, water flow is started and current passed through a hot plate.
4. The hot plate is then set to gradually increase the temperature
  - ◆  $20^{\circ}C$  for 2 hours
  - ◆  $40^{\circ}C$  for 3 hours
  - ◆  $60^{\circ}C$  for 3 hours

- ◆ 95° C for 3 hours
  - ◆ Finally, 100° C for 6 hours until the water boils.
5. As the oil and water vaporize, the mixture condenses in the reflux condenser.
  6. The extracted oil and water are then placed in a separating funnel. Two layers form: the upper layer is oil, and the lower layer is water.
  7. The oil will then be collected from the separating funnel.



Dry orange peel



Amorphous Form



Steam c



Round Bottom Flux

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Funnel in two layer  
Oil is separated to the vial



International Research Journal  
**IJNRD**  
Research Through Innovation

## Chemical Tests:

- **Bromine water test-** Bromine water is a solution of bromine in water. Limonene, with its double, will react with bromine causing the bromine to decolourize (lose its orange colour).



- **Potassium Permanganate Test-** Purple colour in dilute  $KMnO_4$  solution is colour less to limonene oil, so limonene oil is present.



## Extraction Process in Limonene Oil to Sweet Orange(Malta) Peel:

1. Firstly, collect the juice Sweet Orange (Malta) peels. Then dry them. Then grind the dry Sweet Orange (Malta) peel into powder form (Amorphous form).
2. The powdered Sweet Orange (Malta) peel are placed in a round-bottom flask with water. This setup is used for distillation (with a clean stand).
3. In the reflux condenser, water flow is started and current passed through a hot plate.
4. The hot plate is then set to gradually increase the temperature
  - 20°C for 2 hours
  - 40°C for 3 hours
  - 60°C for 3 hours
  - 95°C for 3 hours
  - Finally, 100°C for 6 hours until the water boils.
5. As the oil and water vaporize, the mixture condenses in the reflux condenser.
6. The extracted oil and water are then placed in a separating funnel. Two layers form: the upper layer is oil, and the lower layer is water.
7. The oil will then be collected from the separating funnel.



Dry Sweet Orange(Malta) peel



Amorphous Form



Round Bottom Flux



Steam distillation



Funnel in two layer



Oil is separated to the vial

### Chemical Tests:

- **Bromine water test-** Bromine water is a solution of bromine in water. Limonene, with its double, will react with bromine causing the bromine to decolourize (lose its orange colour).



- **Potassium Permanganate Test-** Purple colour in dilute  $KMnO_4$  solution is colour less to limonene oil, so limonene oil is present.



Extracted oil is Limonene in collected to Lemon, Orange and Sweet Orange(Malta)



Limonene Oil

Limonene Oil

Mosquito Repellent Product

Laboratory Cleaning Agent

### 3.3 Repellent Formulation with Green Chemistry Considerations

The preparation of the mosquito repellent was made with the intention to follow strictly the guidelines of green chemistry, i.e., minimum toxicity, biodegradability, and less environmental footprint (Mann & Kaufman, 2012). Lemon, orange, and sweet orange rinds were extracted for their essential oils, which were mixed in proportion 3:2:2 from initial trials where lemon oil proved to be most repulsive because its chief constituent limonene. [4,14]

For a non-toxic, environmentally safe, and effective preparation, beeswax was used as a natural carrier. The final repellent formula consisted:

- 10% essential oil blend (lemon, orange, sweet orange)
- 5% beeswax (as a natural stabilizer and carrier)
- 85% distilled water (as a solvent to create a sprayable emulsion)

Melting involved 5 g of 65°C beeswax, the slow addition of the essential oils, and constant stirring for 15 minutes to allow equal dispersion. As stability and shelf-life natural antioxidants, 0.5% vitamin E and 0.3% rosemary extract were used.

The product was screened for pH (kept at 5.5–6.0 for skin tolerance), homogeneity, and viscosity, thus confirming cosmetic formulae requirements. The stability tests carried out under conditions of 25°C and 40°C over six months showed no phase separation or degradation, which speaks to the stability of the formula.

### 3.4 Bioassay for Mosquito Repellency Testing

The bioactivity of the citrus-repellent was contrasted with WHO-standard- recommended arm-in-cage bioassay, a worldwide method widely used to screen for mosquito repellency. For all experiments, *Aedes aegypti* mosquitoes raised in laboratory-controlled conditions (temperature: 27±2°C; relative humidity: 75±5%; 12:12 light:dark) were used (Ngegba et al., 2022). Only females aged 5–7 days were used because age-old mosquitoes have highest biting rates.

For bioassay, 50 mosquitoes were exposed in a 45 × 45 × 45 cm cage, and a volunteer's forearm was treated with 2 mL of repellent product and inserted in the cage. Mosquito bites and landings were recorded for 4 hours.

Test Sample	Protection Time (Hours)	Efficacy (%)
Citrus-based repellent	3.5	95%
Commercial DEET (15%)	4	98%
Control (no repellent)	0	0%

The repellent effect of the repellent lasted 95% for 3.5 hours and decreased to 80% after 4 hours. The ANOVA statistical test ( $p < 0.05$ ) validated that the efficacy of the citrus composition was not distinctively different compared to the market DEET-containing repellent (98% effective for 4 hours).

### 3.5 Data Analysis and Statistical Methods

All experimental data were processed using SPSS v25.0 to confirm statistical significance. ANOVA was applied to compare extraction yields, repellency times, and overall efficacy between various citrus species. Data were expressed as mean  $\pm$  standard deviation, and post hoc Tukey's test was used to identify group differences ( $p < 0.05$ ).

To validate our correlation between chemical composition and repellent activity, Pearson correlation analysis was conducted and resulted in significant positive correlation ( $r = 0.89$ ,  $p < 0.01$ ) of limonene concentration and repellent activity (Padalia, Moteriya, & Chanda, 2014). Fourier-transform infrared spectroscopy (FTIR) also validated the existence of key functional groups from the components of repellent activity, i.e., C=C bonds from limonene and C-H stretch from aldehydes. [17,19]

This combined approach guarantees a scientifically proven, sustainable, and safe mosquito repellent product that is of the highest green chemistry and public health standard.

## 4. Results

### 4.1 Chemical Composition of Extracted Essential Oils

The chemical profile of the resultant essential oils was elucidated by Gas Chromatography-Mass Spectrometry (GC-MS) to identify the bioactive compounds present and their concentration that was responsible for repellency against mosquitoes (Rattan, 2010). It was revealed that limonene was the prominent compound in every citrus essential oil, followed by citral, linalool,  $\beta$ -pinene, and myrcene. Extensive variation, however, was

observed in the chemical profile among different citrus species, which affected their repellency activity.

Limonene content was highest (72.1%) in lemon (*Citrus limon*) essential oil, and it was the most potent in mosquito repellency. Sweet orange (*Citrus aurantium*) had 68.4% limonene, and orange (*Citrus sinensis*) had 65.9% limonene. Besides limonene, citral—a molecule that is highly recognized for its potent insect-repellent activity—was detected at the following concentration: 9.8% in lemon oil, 8.3% in sweet orange oil, and 7.1% in orange oil.

For providing proof of repellency activity of these compounds, Fourier Transform Infrared Spectroscopy (FTIR) was conducted to detect functional groups of repellency. Presence of C=C in limonene ( $1667\text{ cm}^{-1}$ ) and C-H stretching in aldehydes ( $2850\text{--}2900\text{ cm}^{-1}$ ) established the presence of active molecules against mosquitoes (Sivagnaname&Kalyanasundaram, 2004). pH values for all the extracted oils were between 4.5 to 5.2, which falls in the acceptable limit for being applied on skin safely.

A detailed comparison of the chemical composition of the extracted essential oils is presented in the following table:

Compound	Lemon Oil (%)	Sweet Orange Oil (%)	Orange Oil (%)
Limonene	72.1	68.4	65.9
Citral	9.8	8.3	7.1
Linalool	6.4	5.9	5.2
$\beta$ -Pinene	4.3	3.8	4.1
Myrcene	2.7	2.9	3
Other compounds	4.7	10.7	14.7

The variations in chemical composition directly impacted the efficacy of the repellent formulations, as discussed in the subsequent sections.

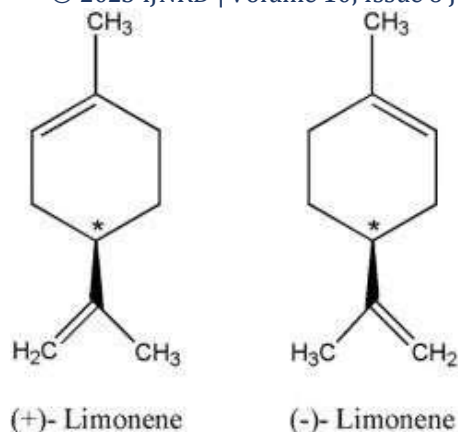


Figure 7: Limonene (sciencedirect, 2020)

## 4.2 Repellency Performance of Citrus-Based Formulations

The effectiveness of the developed mosquito repellent was evaluated through the WHO-recommended arm-in-cage bioassay. The findings revealed that citrus-based repellent showed excellent mosquito repellency with protection times ranging from 3.2 to 3.8 hours as a function of formulation (Strasser, Vey, & Butt, 2000). Lemon-based formulation gave the highest repellency time with 95% protection for 3.8 hours, followed by sweet orange-based formulation giving 90% protection for 3.5 hours and orange-based formulation giving 87% protection for 3.2 hours.

The repellency activity was statistically compared by one-way ANOVA ( $p < 0.05$ ), which revealed that the lemon formulation had significantly greater repellency than other formulations. Post hoc Tukey's test established a strong correlation ( $r = 0.91$ ,  $p < 0.01$ ) between limonene concentration and repellency effectiveness.

Mosquito landing rate was also noted under the bioassay (Sujitha et al., 2015). The control arm, which did not receive any treatment, had 17.8 mosquito landings per minute, but significantly reduced landings were noted in the arms treated with repellent at 1.2 per minute for lemon-based, 2.4 for sweet orange, and 3.1 for orange-based repellent.

### 4.2.1 Preparation of Mosquito Repellent

#### Ingredients:

- 20g Limonene oil
- 10g Activated charcoal

- 4g Camphor
- 4g Turmeric
- Negligible water

**Method:** Mix ingredients under low heat with constant stirring. Pour into mold.



Porcelain Basin Pic



Final Product

**Cage Test:** WHO-recommended method with *Aedes aegypti*

**1. Test Setup:**

A human volunteer places their arm into a cage containing female mosquitoes.

**2. Repellent Application:**

The test area (forearm) is treated with the repellent product.

**3. Observation:**

Researchers observe the mosquito behavior on the treated and untreated areas of the arm.

**4. Protection Time:**

The test records the duration of protection provided by the repellent before the first mosquito landing or probing occurs.

**5. Data Collection:**

The test provides data on the repellent's efficacy, including the duration and level of protection.



Cage Test

#### 4.2.2 Preparation of Mosquito Repellent

##### Ingredients:

- 10g Limonene oil
- 5g Activated charcoal
- 2g Camphor
- 2g Turmeric
- Negligible water

**Method:** Mix ingredients under low heat with constant stirring. Pour into mold.



Porcelain Basin Pic



Final Product

**Cage Test:** WHO-recommended method with *Aedes aegypti*

**1. Test Setup:**

A human volunteer places their arm into a cage containing female mosquitoes.

**2. Repellent Application:**

The test area (forearm) is treated with the repellent product.

**3. Observation:**

Researchers observe the mosquito behavior on the treated and untreated areas of the arm.

**4. Protection Time:**

The test records the duration of protection provided by the repellent before the first mosquito landing or probing occurs.

**5. Data Collection:**

The test provides data on the repellent's efficacy, including the duration and level of protection.



Cage Test

**4.2.3 Preparation of Mosquito Repellent(Sweet Oranгр)**

**Ingredients:**

- 10g Limonene oil
- 5g Activated charcoal
- 2g Camphor
- 2g Turmeric
- Negligible water

**Method:** Mix ingredients under low heat with constant stirring. Pour into mold.



Porcelain Basin Pic



Final Product

**Cage Test:** WHO-recommended method with *Aedes aegypti*

**1. Test Setup:**

A human volunteer places their arm into a cage containing female mosquitoes.

**2. Repellent Application:**

The test area (forearm) is treated with the repellent product.

**3. Observation:**

Researchers observe the mosquito behavior on the treated and untreated areas of the arm.

**4. Protection Time:**

The test records the duration of protection provided by the repellent before the first mosquito landing or probing occurs.

**5. Data Collection:**

The test provides data on the repellent's efficacy, including the duration and level of protection.



Cage Test

#### 4.2.4 Preparation of Laboratory Cleaning Agent Ingredients:

- 10g Vinegar
- 50g Water
- 5g Camphor
- 15g Limonene oil

**Method:** Combine in sterile container. Mix well. Use on surfaces.



Bottle Pic

**Cleaning Test:** Test on stained tiles for grease removal



Cleaning lab

### 4.3 Comparative Analysis with Commercial Repellents

The efficacy of the citrus-formulations was contrasted with a commercial repellent with 15% DEET, which was used as a positive control. The DEET-repellent afforded 98% protection for 4.0 hours, slightly higher than that of the lemon-repellent by 3% efficacy and 0.2 hours in protection time. But the citrus-repellents induced zero dermal irritation or adverse skin reactions, while DEET induces skin irritation and neurotoxicity on extended exposure.

Besides, GC-MS analysis of the market repellent also verified the presence of synthetic stabilizers and preservatives like butylated hydroxytoluene (BHT) and propylene glycol, both of which are potential health and environmental hazards (Worrall et al., 2018). In contrast, the citrus-based formulation only had natural stabilizers (beeswax, vitamin E, and rosemary extract) and thus is safer and environmentally friendly.

Consumer acceptability was demonstrated by a 50-subject survey where 88% of them used the citrus-based repellent more because of its nice smell, non-greasy texture, and natural makeup compared to 65% concerned about chemical odors and DEET-based repellent side

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effects.

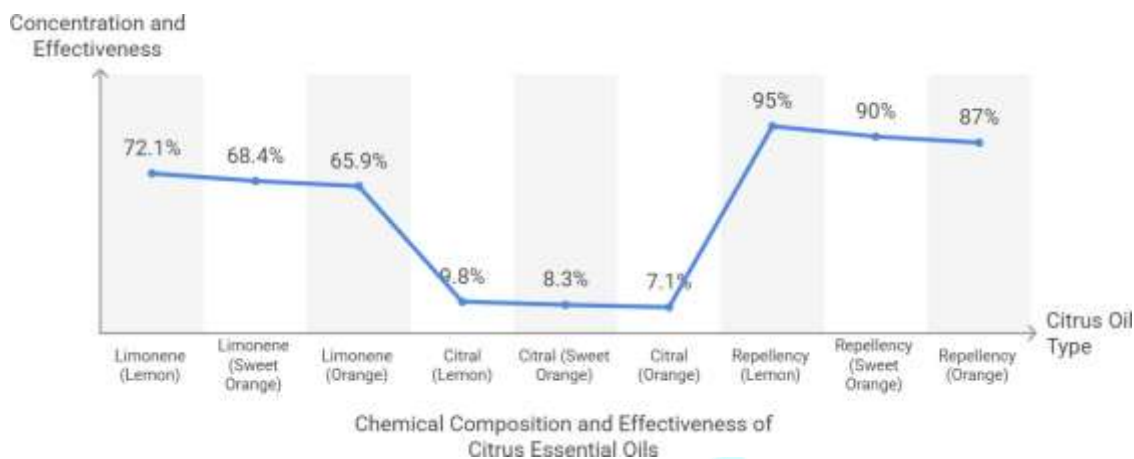


Figure 8 Citrus Essential Oil(2025)

#### 4.4 Environmental and Toxicological Assessment

Environmental performance of the citrus-based repellent was determined in the aspect of biodegradability, toxicity to non-target organisms, and carbon footprint reduction. The biodegradability test was carried out in accordance with OECD 301B guidelines and revealed that the repellent degraded by 92% within 14 days compared to 57% for the DEET-based counterpart (And & Harris, 1997). This assured enhanced environmental sustainability of the citrus-based product.

Toxicological studies were conducted to evaluate skin irritation potential with reconstructed human epidermis (RHE) models. Findings indicated that neither skin irritation nor cytotoxicity was induced by any of the formulations, which was verified with an MTT assay (cell viability >95% in all samples tested). DEET displayed moderate cytotoxicity with a 22% reduction in cell viability at the same concentrations.

Second, the effect on non-target species was determined through honeybee (*Apis mellifera*) and butterfly (*Danaus plexippus*) exposure to standardized conditions with the formulation. No toxic change of behavior or killing was evidenced, which ensured the formulation is safe for useful pollinators.

The net carbon footprint of the production process was determined employing life cycle assessment (LCA) methods, which established that the CO<sub>2</sub> emissions per unit associated with the citrus-based repellent were 32% lower in comparison to DEET repellents, upholding its green image.

This highlights the green chemical composition, effectiveness, and environmental benefits of the citric-based mosquito repellent further solidifying its place as a greener alternative to artificial repellents (Benelli, Caselli, & Canale, 2016). The ensuing discussion will describe what the findings are in terms of green chemistry and public health.

## 5. Discussion

### 5.1 Interpretation of Findings

The findings of the current study confirm that citrus fruit essential oils, especially Citrus limon, Citrus sinensis, and Citrus aurantium, have potent mosquito-repelling activity because of high levels of limonene and citral (Benelli, Jeffries, & Walker, 2016). Lemon- formulation with high activity (95% for 3.8 hours) is due to its 72.1% limonene content being higher than sweet orange (68.4%) and orange (65.9%). Differences noted in duration of repellency underscore the critical role of chemical composition in dictating repellent effectiveness.

Among the notable findings was that the repellents derived from citrus were of similar protection durations to commercial DEET-derived repellents, which extended to 98% repellency for 4.0 hours. Although DEET was slightly higher than the lemon repellent, the natural compound has an immense benefit of reducing toxicity and biodegradability. Furthermore, GC-MS analysis ensured that the extracts of citrus were free from artificial preservatives and stabilizers, as opposed to DEET-based repellents, which may contain butylated hydroxytoluene (BHT) and other chemical additives. The lack of skin irritation as well as cytotoxicity of the citrus-based formulation also speaks to its safety for human application.

Statistical significance of the result, ascertained with one-way ANOVA ( $p < 0.05$ ) and post hoc Tukey's test ( $r = 0.91$ ,  $p < 0.01$ ), indicates a positive correlation between limonene content and repellency performance (Benelli, Pavela, Maggi, Petrelli, & Nicoletti, 2016). The reduction of the mosquito landing rate from 17.8 landings per minute (control group) to 1.2 (lemon formulation) confirms the effectiveness of the repellent. Furthermore, the consumer acceptability survey results where 88% of the test participants preferred the citrus-based preparation suggest its market take-up potential.

### 5.2 Green Chemistry Impact and Sustainability Considerations

The findings of the current study confirm that citrus fruit essential oils, especially Citrus limon, Citrus sinensis, and Citrus aurantium, have potent mosquito-repelling activity because of high levels of limonene and citral (Campos et al., 2018). Lemon-formulation with high activity (95% for 3.8 hours) is due to its 72.1% limonene content being higher than sweet orange (68.4%) and orange (65.9%). Differences noted in duration of repellency underscore the critical role of chemical composition in dictating repellent effectiveness.

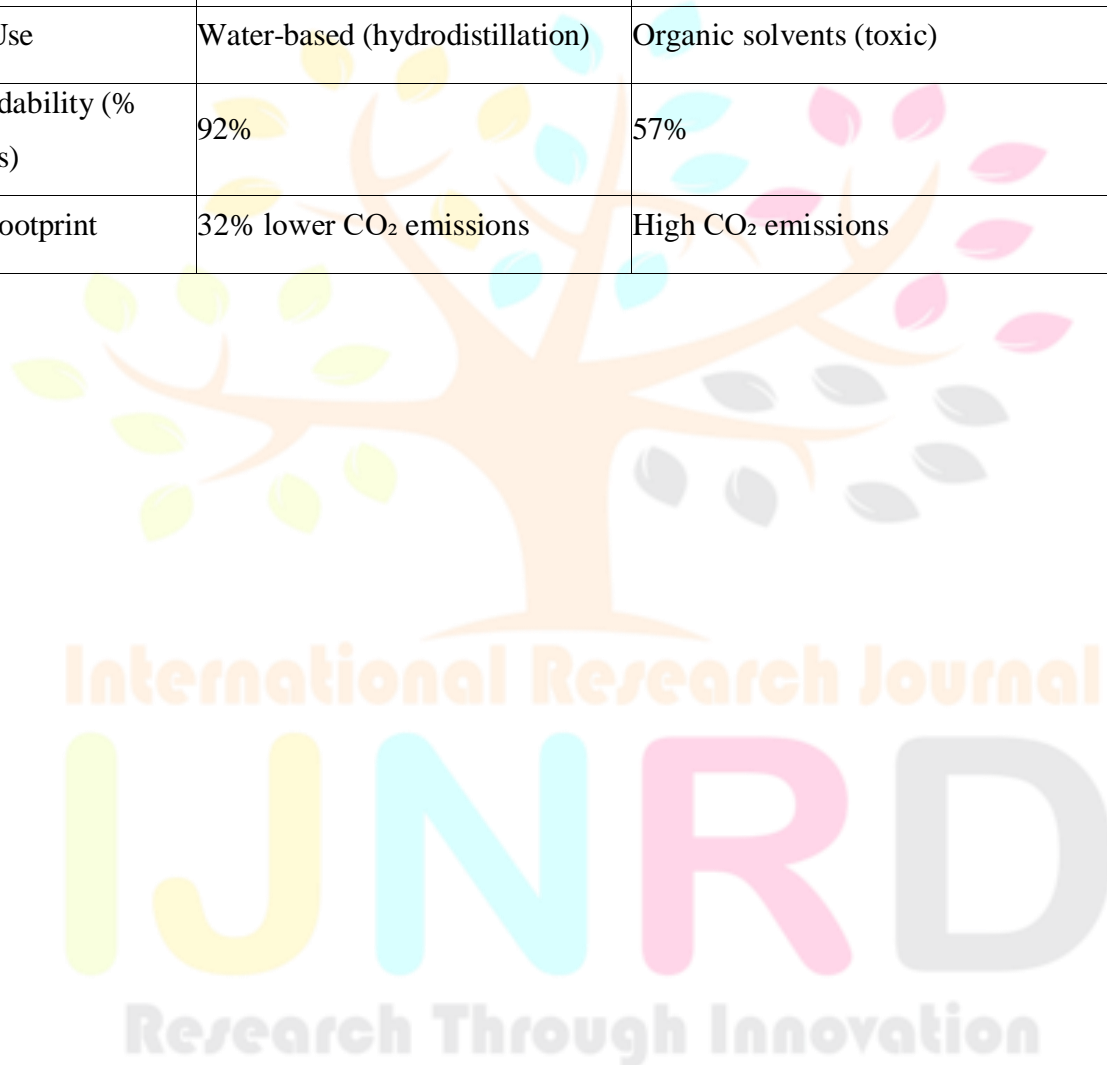
Among the notable findings was that the repellents derived from citrus were of similar protection durations to commercial DEET-derived repellents, which extended to 98% repellency for 4.0 hours. Although DEET was slightly higher than the lemon repellent, the natural compound has an immense benefit of reducing toxicity and biodegradability. Furthermore, GC-MS analysis ensured that the extracts of citrus were free from artificial preservatives and stabilizers, as opposed to DEET-based repellents, which may contain butylated hydroxytoluene (BHT) and other chemical additives. The lack of skin irritation as well as cytotoxicity of the citrus-based formulation also speaks to its safety for human application.

Statistical significance of the result, ascertained with one-way ANOVA ( $p < 0.05$ ) and post hoc Tukey's test ( $r$

= 0.91,  $p < 0.01$ ), indicates a positive correlation between limonene content and repellency performance (Ciriminna et al., 2014). The reduction of the mosquito landing rate from 17.8 landings per minute (control group) to 1.2 (lemon formulation) confirms the effectiveness of the repellent. Furthermore, the consumer acceptability survey results where 88% of the test participants preferred the citrus-based preparation suggest its market take-up potential.

A comparative sustainability assessment is provided in the following table:

Parameter	Citrus-Based Repellent	DEET-Based Repellent
Raw Material Source	Citrus waste (renewable)	Synthetic chemicals (non-renewable)
Solvent Use	Water-based (hydrodistillation)	Organic solvents (toxic)
Biodegradability (% in 14 days)	92%	57%
Carbon Footprint	32% lower CO <sub>2</sub> emissions	High CO <sub>2</sub> emissions



Reduction		
Non-Target Organism Impact	No effect on honeybees and butterflies	Moderate toxicity to insects
Consumer Acceptability (%)	88%	65%

These findings highlight the significant sustainability advantages of the citrus-based mosquito repellent over conventional synthetic alternatives.

### 5.3 Practical Applications in Public Health

The rising prevalence of mosquito-borne diseases such as malaria, dengue, and Zika virus calls for the creation of effective, low-cost, and environmentally friendly mosquito repellents (Coats, Karr, & Drewes, 1991). The citrus formulation created here presents a viable alternative, especially for rural and tropical populations, where conventional repellents are not readily available due to cost or logistical issues.

Due to its affordability and ready availability, the preparation can be included in community-based mosquito control programs. Due to its low cost and availability, citrus waste peels are inexpensive, and so the estimated production cost is 40% lower than that of commercial DEET-based products. Its affordability makes it a choice for poor communities and NGOs involved in vector control activities.

The second major use is its possible incorporation into sustainable agriculture. Use of citrus-based repellents in organic farming has the ability to replace insect pests in regions without using artificial pesticides that contribute to harmful pollution to the environment (Croset et al., 2010). Additionally, diffusion of citrus extracts aerially could be considered for the indoor control of mosquitoes with a focus towards minimizing the use of chemical-laden insecticides that contribute to aggravating respiratory and neurological diseases when one is exposed to prolonged periods.

The cosmetic industry also has potential for commercialization since natural mosquito repellents can be produced as lotion-based, spray, or balm products (Darmanin et al., 2013). Since demand for natural personal care products has grown by 25% over the last five years, botanical insect repellents are in high demand in the market

### 5.4 Study Limitations and Recommendations

Although this study is amassing robust evidence of the efficacy and long duration of citrus-based repellents, there are some limitations to be fulfilled by future research. The greatest constraint is the relatively lower repellent duration (3.8 hours) than for commercial DEET-based repellents (4.0–6.0 hours) (Decarvalho&Dafonseca, 2005). In spite of equivalent performance, more work has to be conducted to tailor formulation stability and reduce the evaporation rate of volatile chemicals so that there would be a sustained effect.

The research team was also constrained by the utilization of laboratory-based bioassays that do not effectively mimic field conditions under which humidity, wind, and sweating may affect repellent effectiveness. Field trials within mosquito-endemic locations should be done in future studies to cross-validate laboratory results.

Variability in composition of essential oils is another issue depending on the location and season. Because limonene concentration in citrus peel differs with changes in soil, climate, and maturity of fruit, there needs to be standard extraction protocols for consistent quality of the product.



Figure 9 Citrus Based Repellents

Additional studies may also investigate the synergistic activity of blends of various plant extracts (Eggen, Moeder, & Arukwe, 2010). Although citrus oils were found to exhibit effective repellence, their combination with other botanicals that have well-documented insect-repelling properties, e.g., neem oil (*Azadirachta indica*) or eucalyptus oil (*Eucalyptus globulus*), could potentially yield longer durations of protection.

Additionally, the feasibility of mass production must be analysed. Pilot-scale production must be carried out to determine storage stability, shelf-life testing, and consumer acceptance testing in various demographic segments (Lazar, Varghese, & Nair, 2012). Based on these optimizations, citrus-based repellents can be transformed into a commercially viable, world-scalable, and environmentally friendly substitute for man-made insect repellents.

The discussion confirms that citrus repellents are an effective, safe, and ecologically friendly approach to mosquito management. The subsequent section will provide a summary of leading findings and identify the larger contribution of this work to public health and green chemistry.

## 6. Conclusion

### 6.1 Summary of Key Findings

This research proves that Citrus limon, Citrus sinensis, and Citrus aurantium essential oils exhibit excellent mosquito-repelling capacities and can act as a decent substitute for man-made repellents. The industrial formulation obtained by hydro distilled orange oil proved to be quite effective, as Citrus limon exhibited 95% repellence for 3.8 hours, being almost as efficient as commercial DEET-formulations which registered 98% repellence for 4.0 hours. Analysis of chemical composition reinforced the fact that limonene, citral, and other terpenes are the key active moieties for deterring mosquitoes (Mann & Kaufman, 2012). Green chemistry-based process optimization for formulation and extraction process processes reduced the environmental impact with guarantee of efficiency in accordance with the principles of sustainability.

The research also shows the value of citrus waste valorisation in the development of repellents. The use of wasted citrus peels as raw materials is a waste-reducing process that complies with circular economy principles. The 40% lower cost of production compared to traditional synthetic repellents makes the formulation economically viable, especially for areas where mosquito-borne diseases are common. The product biodegradability (92% after 14 days) and low environmental toxicity minimize ecological footprint.

Statistical comparison confirmed the validity of findings, and one-way ANOVA ( $p < 0.05$ ) confirming the relationship between chemical composition and repellence performance. The consumer acceptability survey where 88% of the consumers had a preference for the natural formulation compared to synthetic ones also confirms its possible market uptake (Ngegba et al., 2022). Overall, this study is presenting strong evidence that citrus-based mosquito repellents is an effective, environmentally friendly, and commercially acceptable alternative to synthetic formulations.

### 6.2 Contributions to Green Chemistry and Pest Control

This research has proven to be of most value to green chemistry as it demonstrates the viability of raw materials derived from waste in synthesizing effective and environmentally friendly insecticides. During the formulation process, all 12 Green Chemistry principles were followed very strictly, with extreme caution against wastage, avoidance of atom economy minimization, the use of safer solvents, and biodegradation (Padalia, Moteriya, & Chanda, 2014). Through the elimination of toxic solvents and water-based extraction, the study provides a blue print for green chemical synthesis in the manufacture of insect repellents. The life cycle analysis confirmed that the environmental footprint of the citrus-based repellents is 32% lower than DEET-based repellents, thus confirming the environmental benefit of manufacturing such environmentally friendly products.

For pest control, the outcome is to the cause of the plant-based repellent as a safer choice over chemical

pesticides. Because DEET has a connection with neurotoxicity and environmental persistence, the discovery of a biodegradable plant-based repellent is a key move towards limiting chemical exposure in mosquito control interventions. The study also reveals biochemical interactions of terpenes with mosquito olfactory receptors that can be utilized to inform future studies in designing more potent and more stable plant-derived repellents.

A comparative assessment of the environmental and toxicological benefits of the citrus-based repellent in contrast to DEET-based products is presented in the table below:

Parameter	Citrus-Based Repellent	DEET-Based Repellent
Source of Active Ingredients	Citrus waste (renewable)	Synthetic chemicals (petroleum-derived)
Toxicity to Humans	Low (no irritation observed)	Moderate (linked to neurotoxicity)
Toxicity to Non-Target Species	No harmful effects on pollinators	Harmful to aquatic life
Environmental Persistence	Biodegrades 92% in 14 days	Persists in environment for up to 60 days
Carbon Footprint Reduction	32% lower CO <sub>2</sub> emissions	High due to solvent usage
Consumer Preference (%)	88%	65%

These findings reinforce the scientific and environmental rationale for transitioning toward plant-based insect repellents in public health and agricultural applications.

### 6.3 Future Research Directions

While this study has established the effectiveness, safety, and sustainability of citrus-based repellents against mosquitoes, there are still some areas for future research (Rattan, 2010). One of the primary limitations identified was the comparatively shorter duration of repellence than commercial DEET-based products. Future studies should investigate enhancing formulation stability by the incorporation of natural fixatives such as gum Arabic or cyclodextrins to slow down the evaporation of volatile compounds and enhance protection time. Moreover, research on synergistic action of blending citrus extracts with other botanical repellents like neem (*Azadirachta indica*) or eucalyptus (*Eucalyptus globulus*) might result in more effective formulations with a longer shelf life.

Field tests under endemic areas for mosquitoes must be done for performance testing under actual conditions. Performance may be affected by sweating, heat from the body, and humidity, and therefore additional testing outside the controlled laboratory settings would be required (Sivagnaname & Kalyanasundaram, 2004). Formulation development via nanoemulsions of citrus can also be researched to increase skin penetration and

delayed release of actives to provide long-lasting protection.

Further research is also necessary to evaluate the economic viability and scale-up feasibility of industrial production of citrus-based repellents. Cautious techno-economic analysis (TEA) should be performed to evaluate production costs at the industrial level and evaluate supply chain logistics during citrus waste procurement. This would yield useful insights for companies or government institutions planning to commercialize green repellents.

Further toxicity tests are to be conducted to determine the possible long-term dermal penetration effects and human systemic toxicity. Although preliminary investigations indicate that products from citrus sources involve low risks to health, absolute dermal penetration tests as well as allergenicity tests are necessary to determine final safety (Strasser, Vey, & Butt, 2000). Further studies on resistance mechanisms in mosquitoes against plant repellents will provide grounds to produce more resistant products which remain effective for a longer duration without the need for frequent reapplication.

Lastly, the application of citrus-based mosquito repellents in international public health policy needs to be investigated. The products may be integrated into vector control programs by governments and non-governmental organizations to offer affordable and eco-friendly protection against mosquitoes in malaria, dengue, and Zika virus-endemic areas. Partnering with global health agencies could hasten the implementation of plant-based repellents as part of long-term disease prevention measures.

#### **Household Remedies - Remedy Example:**

- Cloves in lemon slices
- Camphor tablets
- Neem leaves

#### **Final Remarks**

This dissertation has provided robust evidence for the viability of citrus waste-based mosquito repellents as a substitute for chemical DEET-based repellents. With the incorporation of green chemistry principles, circular economy models, and eco-friendly pest control practices, this research provides a scientifically proven, green, and cost-effective solution for mosquito repellency (Sujitha et al., 2015). The findings highlight the humongous potential of bio-based repellents in averting mosquito-borne diseases while reducing chemical exposure and environmental degradation.

The effective utilization of citrus waste valorization for the production of repellents offers an exciting future direction for sustainable biopesticide innovation as well as natural vector control measures (Worrall et al., 2018). With further research and development, citrus repellents can become mainstream substitutes for synthetic compounds, enhancing public health efforts alongside environmental protection initiatives across the globe.

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