

Eco-Friendly Mosquito Repellent; Plant based Larvicide against *Aedes aegypti*

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Abstract

Global health continues to be seriously threatened by vector-borne diseases, which are mostly spread by arthropods like mosquitoes, especially in tropical and subtropical areas. A frightening outbreak of dengue fever, which is caused by the dengue virus (DENV), occurred throughout the Americas in 2023, with over 2.9 million cases reported. Chemical, biological, and environmental approaches are being used in vector control tactics; however, the use of synthetic pesticides such as DDT and pyrethroids has resulted in ecological problems like as resistance, environmental contamination, and damage to creatures that are not the intended target. Behavioral adaptations, physiological alterations, and decreased cuticle penetration are among the resistance mechanisms in *Aedes aegypti*, the main vector of dengue. New environmentally friendly substitutes, such bio-insecticides made from phytochemicals, present encouraging options. These medicines have decreased toxicity and stop bioaccumulation, and they target mosquitoes with little effect on the environment or human health. Effective lethal concentrations (LC₅₀ values) are a sign of the potential of larvicidal plants, which include species from the Fabaceae, Asteraceae, and Piperaceae families. Mosquito populations have been shown to be effectively reduced by essential oils and plant extracts, such as those from *Mentha piperita* and *Ocimum gratissimum*. The study highlights the significance of sustainable vector control methods that use plant-based bio-insecticides to solve ecological issues and vector resistance while reducing the drawbacks of synthetic pesticides.

Keywords: Mosquito repellent, Natural larvicides, *Aedes aegypti*, Resistance, Eco-Friendly alternatives, Toxicity, Pyrethroids, vector control, Dichlorodiphenyltrichloroethane (DDT), Essential oils, Biodegradable alternatives

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Introduction

Vector-borne diseases refer to infections caused by arthropod pathogen transmission. Vector-borne illnesses are infections caused by arthropods transmitting pathogens. Arthropods such as mosquitoes can transmit vector-borne diseases (Kamaraj et al., 2010; Wilson et al., 2020). In five WHO regions—Africa, the Americas, Southeast Asia, the Western Pacific, and the Eastern Mediterranean—more than 100 nations and territories are currently reporting more than 5,000 deaths from DENV (Kuo et al., 2024). In 2023, the Americas witnessed a major dengue fever outbreak, with 2,997,097 cases reported between epidemiological weeks 1 and 26. Brazil had the most cases (2,376,522), followed by Peru (188,326) and Bolivia (133,779). The cumulative incidence rate was 305 cases per 100,000 persons, with a case fatality rate of 0.04 percent. Severe dengue cases have been observed, mainly in Colombia and Mexico (Akinsulie & Idris, 2024). The rise of mosquito-borne arboviruses raises worries about public health and the potential for worldwide disease outbreaks (Huang et al., 2019). Dengue fever shares clinical features with coronavirus illnesses (COVID-19) (Wu et al., 2020). Current disease vector control approaches fall into three categories: environmental, chemical, and biological. Environmental safeguards include long-sleeved clothing, bed nets, and covering water-prone items like tires and jars. Vector control strategies include using mosquito coils, repellent creams or lotions, temephos, and insecticidal sprays (Shafique et al., 2019). Biological vector control approaches mostly use microbes such as *Bacillus thuringiensis israelensis*, guppy fish, and sterile insects (Hamed et al., 2022). Although vaccination is a vector control method, it has been found to be ineffective against dengue illnesses (Redoni et al., 2020). Pyrethroids are the most common class of insecticides used for vector control. This is because of a number of their characteristics, including their limited capacity to harm mammals and great toxicity to target vectors. Since pesticides continued to be the primary means of controlling the mosquito vector, *Aedes aegypti*, inside endemic areas, pyrethroids have been used extensively throughout the past thirty years (Smith et al., 2018). Synthetic pesticides can lead to insecticide resistance, harm to non-target creatures, and environmental contamination, among other ecological issues (Manorenjitha Malar et al., 2017). Bio-insecticides offer several benefits for vector control. Bio-insecticides, for example, offer a unique mode of action that allows them to target certain vectors while avoiding harm to non-target organisms. Bio-insecticides can prevent bio-accumulation in soils due to their biodegradable qualities. Biodegradable materials can reduce environmental pollution. Aside from that,

these target vectors have a very low likelihood of developing resistance to specific insecticides. Bio-insecticides are a preferred alternative to chemical insecticides for vector control (Prasannath, 2016).

1. Insight to Dengue and *Aedes aegypti*

Dengue fever is a viral disease transmitted through vectors and caused by the flavivirus dengue virus. More than 100 tropical and subtropical nations have recorded cases. *Aedes* mosquitos are primarily responsible for transmitting the positive-stranded enveloped RNA virus (DENV). Each of its four antigenically distinct serotypes—DENV-1 through DENV-4—has three structural proteins, seven nonstructural proteins, and a unique genotype. Clinical signs and symptoms of dengue range from mild fever to severe dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), which involves increased vascular permeability, leucopenia, and thrombocytopenia. Heterotypic infection with various serotypes and antibody-dependent enhancement (ADE) both increase the severity of the disease, even while primary infection triggers immune responses against DENV serotypes (Roy & Bhattacharjee, 2021). Female *Aedes* mosquitoes can spread the dengue virus, and humans are the primary hosts for dengue illnesses. Normally, the female mosquito becomes infected during the blood feeding phase. During incubation, the virus enters mosquito midgut cells and replicates within tissues. After 8-10 days, mosquito salivary glands become infected and can transmit dengue virus to the host (Guzman et al., 2016). Dengue is classified into three types: fever, hemorrhagic fever, and shock syndrome (Oishi et al., 2007). While numerous medicines and drugs are pushed for treating dengue, their efficiency in curing the disease remains poor (Subenthiran et al., 2013). There are no therapeutic medications or therapies to completely cure dengue illness (Subenthiran et al., 2013; Zaheer et al., 2022).

Mosquitoes belong to the *Culicidae* family of the order *Diptera* and suborder *Nematocera* (Wilkerson et al., 2015). Mosquito variety can be influenced by various circumstances, including mating regions, resting and reproductive sites, and sources of nectar and blood (Diallo et al., 2012). Evolution within a mosquito's life is called metamorphosis. They have a four-stage life cycle: egg, larva, pupa, and adult. Mosquito egg-laying sites are generally needs to be present closer to stagnant water sources. Mosquitos use female to lay eggs and start the growth of the larvae. In the larval stage, food requirements include organic matter and microorganisms. After 7 days, the larvae stage converts every pupa into adult (Rueda, 2008; Faithpraise et al., 2014). A mosquito that is the main vector for dengue sickness is *Aedes aegypti*. These mosquitos love about to bite lower parts of the host body. Most of the time, *Aedes aegypti* feeds in shady places during the day. *Aedes aegypti* mosquitos are highly anthropophilic, mainly feeding on humans. However, it can also eat other animals if they are available (Farajollahi & Price, 2013).

1.1 Role in Transmitting Diseases (Dengue, Zika, Chikungunya)

Mosquito-borne viruses, called arboviruses, have tormented humans for thousands of years and cause tremendous suffering still today. Although not being the only known mosquito-borne viruses, the viruses responsible for yellow fever (YVF), dengue fever (DENV), chikungunya fever (CHIKV) and zika fever (ZIKV) are the virus class with the highest awareness for the high severity of disease and impact on humanity. One mystery surrounding these four viruses is that a single mosquito (out of over 3,500 species), *Aedes aegypti*, has been the vector of practically all significant epidemics outside Africa (Powell, 2018).

2. Traditional Larvicides

2.1 Pyrethroids

Pyrethroids are commonly used pesticides that are known to be neurotoxic. Pyrethrin is an organic chemical that can be derived from the flower *Chrysanthemum cinerifolius* (Davies et al., 2007). Various institutions, including research labs, and agrochemical corporations, have performed extensive study to demonstrate the diverse spectrum of pyrethroid structures. Natural pyrethroid insecticides may lose stability when exposed to air or light (Soderlund, 2012). These natural insecticides have limitations in their ability to effectively control pests and safeguard crops. Thus, synthetic insecticides derived from the original structure of natural pyrethroids can increase stability under light and air conditions. Modified pyrethroids can preserve their original properties, including reduced mammalian toxicity and excellent insecticidal activity. The two synthetic pyrethroid insecticides are permethrin and deltamethrin. Two synthetic pyrethroid insecticides improved insecticidal activity and photostability compared to natural pyrethroids. Pyrethroid pesticides stick to open sodium channels and alter the channel regulating transition. This can lead to longer periods of sodium channel opening (Soderlund, 2012; Du et al., 2016).

Pyrethrins are used in agriculture and pest management, which results in a variety of environmental pollutants that harm human health and a decline in the population of soil microbes that influence soil fertility and health. In addition to producing significant quantities of reactive oxygen species, pyrethroids are known to alter the human plasma biochemical profile. They are also known to raise human SGPT activity. Given the toxicity of pyrethrins in soils, water bodies, and food items, it is imperative to create environmentally benign, financially feasible, and socially acceptable on-site remediation methods to lower their concentrations in the corresponding fields. With this in mind, an effort has been made to examine the developments and opportunities in the use of pyrethrins and potential solutions to limit their negative effects. The study article has covered in fully the varieties of pyrethroid insecticides, their makeup and biochemistry, and their effects on both people and insects. The effect of pyrethroids on various plants and soil microbial flora is also covered (Singh et al., 2022).

2.2 Dichlorodiphenyltrichloroethane (DDT)

A lipid-soluble kind of organophosphate insecticide, dichlorodiphenyltrichloroethane (DDT) is an older variant of the pesticide than others. 1, 1, 1-Trichloro-2,2-bis(p-chlorophenyl) ethane is the scientific name for DDT. DDT is used in certain areas, such as Africa, to address vector control issues, although India is the main industrial nation that produces large amounts of DDT in insecticides (Du et al., 2016).

According to reports, DDT pesticides have neurotoxic qualities that include prolonging the opening times of sodium ion channels and preventing the activation of potassium gates. Additionally, DDT pesticides target certain neural ATPase across the nervous system to change the pace at which components like calcium, potassium, and sodium flow. The primary component that can aid in neurotransmitter release mechanisms is calcium ions, however DDT pesticides can prevent calcium ion transfer. These combined effects can amplify transmitter release,

improve the sustainability of neuron depolarization, and cause CNS excitement, including tremors and heightened vigor (Harada et al., 2016).

DDT pesticides primarily affect the peripheral nervous system. Insecticide exposure can cause neurological symptoms in insects, including quivering, muscular twitching, and tremors of appendages. Increased neurotransmitter release can lead to greater post-synaptic dispersion. Depolarization at neuromuscular connections may result from these events. Neurotransmitter depletion can cause joint inhibition due to ongoing depolarization (Perry et al., 1998; Davies et al., 2007).

3. Challenges in Controlling *Aedes aegypti* Populations

3.1 Resistance

Insecticide resistance is a significant hazard, particularly among mosquitos. Most countries use synthetic insecticides to combat mosquito vectors and slow the spread of vector illnesses. However, using synthetic insecticides for vector control can lead to pesticide resistance and harm non-target organisms. *Aedes* mosquitos (Smith et al., 2018) exhibit resistance to pyrethroid pesticides through cytochrome P450 monooxygenase detoxification and knockdown resistance (kdr). Single and combination alterations in sodium channels with voltage gates are seen in *A. aegypti* mosquitoes.

Knockdown Resistance

Knockdown resistance (kdr) is a common pesticide resistance that targets bugs' sodium channels. Synthetic pesticides such as DDT and pyrethroids can be distributed through target vectors' sodium channels, leading to insect mortality. Insecticide resistance is mostly caused by point mutations in the para-type sodium channel within insect bodies (Bass et al., 2007). Mutations can impair the efficiency of pesticides against target insects. The change of sodium channels can result in a lower efficacy of pesticides against insects that have evolved resistance compared to non-resistant insects. Research indicates that pyrethroids and DDT insecticides are mostly responsible for kdr mutation. Additionally, combining DDT and pyrethroid pesticides might result in cross-resistance and insecticide resistance (Davies et al., 2007; Kushwah et al., 2015).

Behaviour Resistance

Insects typically respond to pesticides through physiological resistance or behavioral avoidance (Chareonviriyaphap et al., 1997; Chareonviriyaphap et al., 2013). Physiological resistance is a type of pesticide resistance that allows certain populations of insects to survive in conditions where insecticide concentrations would typically cause insect mortality (Roberts et al., 1997; Chareonviriyaphap et al., 2013; Carrasco et al., 2019). Combining many physiological resistances remains possible. Physiological resistances can be overcome by enhancing enzyme activity (e.g., P450 mono-oxygenase or glutathione S-transferases) and modifying nerve receptors at specific sites (Ranson et al., 2011). Insects can avoid exposure to insecticides through behavioral avoidance, also known as behavioral resistance. Insects with behavioral resistance can recognize and avoid pesticides in their environment. Its features allow for the detection of environmental changes (Gatton et al., 2013; Panini et al., 2016).

To differentiate them from behavioral resistances, resistance can be divided into four main stages: exploitation, tolerance, quantitative resistance, and qualitative resistance. Mosquitoes exhibit qualitative behavioral resistance, which prevents exposure to pesticides. Mosquitoes can minimize insecticide exposure by timing their activities to avoid clashes with insecticide application timings. Mosquitoes can change their feeding places to lower pesticide exposure rates. In the second phase of behavioral resistance, mosquitos may react to avoid areas with high insecticide concentrations. Exposure to insecticides can be decreased (Carrasco et al., 2019). The third step of resistance is behavioral tolerance, which is suited for mosquitos that cannot escape or reduce the pace of insecticidal effects. Mosquitoes, for example, can change how and where their eggs are produced and distributed. Additionally, resistance can improve blood nutrient quality and reduce energy consumption. Resistance might lead to reorganization in egg distribution and production (Cutler, 2013; Carrasco et al., 2019).

Reduce Cuticle Penetration Resistance

Most insects produce apical extracellular matrix around various body components, including the trachea, epidermis, foregut, and hindgut. Extracellular matrixes called cuticles are created during particular phases, like moulting and metamorphosis. The natural procedure that can produce insect cuticles is called embryogenesis (Moussian, 2010). Insecticide dispersion in hemolymph reduced as penetration through the cuticle decreased. Insecticides' sluggish dispersion in insect hemolymph can delay detoxification. This can limit the buildup of insecticides in particular areas of the insect body system (Strycharz et al., 2013). Modifying the cuticle is essential for developing resistance to penetration. The two key characteristics involved in cuticle alterations are increased cuticle thickness and altered cuticle components. Cuticle thickness has a stronger correlation with pesticide resistance, while other research suggests a relationship between reduced xenobiotic penetration and cuticle composition (Dang et al., 2017; Balabanidou et al., 2018). Insecticide resistance is effective against a wide range of pesticides; however, resistance rates are low. Cuticle penetration resistances can enhance the effectiveness of other pesticide resistances (Strycharz et al., 2013; Kasai et al., 2014; Panini et al., 2016).

4. Eco-friendly Alternatives for Larvicidal Control

Plants evolved many defense mechanisms to protect themselves from natural adversaries. In addition to plants, insects developed to be able to resist plant protective mechanisms. Plants contain various molecules that can provide defense against predators or microorganisms. Plants can use these genetic molecules to guard against vertebrates due of their comparable neural signaling pathways throughout animal kingdoms. Compared to single compounds, combinations of secondary metabolites may have longer-lasting inhibiting effects against pests and herbivores. Furthermore, its physical characteristics may offer plants durable protection (Rattan, 2010).

Phytochemicals with insecticidal qualities found in plants include terpenoids, phenolics, alkaloids, steroids, and essential oils. Unsystematic

use of chemical pesticides causes a variety of severe difficulties. Insects can develop genetic resistance to particular insecticides, rendering them ineffective. Chemical pesticides pose health risks and come at a great expense (Dinesh et al., 2014). For vector management, bio-insecticides have been suggested as a good substitute for chemical insecticides. Bio-insecticides have a few advantages that make them a promising new vector control method. It protects non-target organisms from hazards due to its low toxin content. Bio-insecticides have reduced costs compared to chemical insecticides. Bio-insecticides are safer for users due to their low toxicity. It can slow bio-accumulation and reduce environmental residues. Furthermore, very low quantities of bio-insecticides are required to eliminate target vectors (Ohia & Ana, 2015). Variables like plant section, species, and target affect how effective plant-based bio-insecticides are. The polarity of the solvent and the extraction methods affect the effectiveness of plant-based bio-insecticides (Bharathithasan et al., 2024).

5. Larvicidal Activities of Plants

According to Abbas et al. (2022), a total of 859 publications were reviewed for plant extracts and fatal concentration estimations (LC₅₀ and LC₉₀). Finally, 95 studies describing the larvicidal potential of 150 plant species from 52 families were reviewed. The two most investigated families for this activity were *Fabaceae* and *Asteraceae*. The plant families with the lowest LC₅₀ values against mosquitoes were *Piperaceae* and *Annonaceae*. 50 acetogenins have been found to have larvicidal action on *Aedes aegypti*, with 29 of them having an LC₅₀ below 10 µg/mL. Additionally, 8 compounds isolated from *Piperaceae* had larvicidal activity. (Silva, 2012) believes that compounds with LC₅₀ values (lethal death concentration of 50%) less than 100ppm are effective larvicidal agents.

The study demonstrated that *Ocimum gratissimum*, *Ocimum basilicum*, *Pogostemon heyneanus*, and *Hyptis crenata* have significant larvicidal capability, with LC₅₀ values (ppm) of 76.6, 67.2, 69.9, and 89.4 correspondingly (Ramos, 2014). Shabab Nasir et al. (2015) discovered that *Mentha piperita* is very poisonous to *A. aegypti* mosquito larvae, causing roughly 90% larval mortality.

As reported by (Waliwitiya et al., 2009) Pulegone, thymol, eugenol, trans-anethole, rosemary oil, and citronellal exhibited high larvicidal efficacy against all *Aedes aegypti* larval stages (LC₅₀ values 10.3-40.8 mg L⁻¹). Among the 57 organic extracts tested, *Saussurea lappa*, *Ocimum tenuiflorum*, *Taraxacum officinale*, *Nigella sativa*, and *Hyssopus officinalis* killed over 80% of adult female *Aedes aegypti* at 5 µg/mosquito. In the larvicidal bioassay, the petroleum ether extract of *Aloe perryi* flowers showed 100% mortality against 1st instar *Aedes aegypti* larvae at 31.25 ppm concentration. This investigation assessed the larvicidal and pupicidal properties of the main components and essential oils (EOs) derived from *Zanthoxylum limonella* and *Illicium verum* against *Aedes aegypti* and *Aedes albopictus* mosquitoes (Soonwera et al., 2022).

Conclusion

Aedes aegypti poses significant health hazards, hence sustainable vector management is an essential method. Eco-friendly larvicides, particularly those derived from plants with proven larvicidal action, are a viable alternative to manufactured chemical treatments. These plant-based therapies, which use bioactive chemicals, offer an effective, environmentally friendly, and potentially cost-effective approach of controlling mosquito populations. This chapter has presented scientific, practical, and future-oriented perspectives on these approaches, stressing their importance in disease prevention while safeguarding ecosystems. The next step requires more study into plant species with larvicidal capabilities, such as extracting active chemicals, developing formulation procedures, and testing for long-term efficacy and environmental safety. Botanists, chemists, and entomologists must work together to fully realize the promise of natural solutions. Policymakers have a vital role in closing the gap between research and implementation. This includes promoting legislation that encourage the approval and broad use of plant-based larvicides, as well as investing in research funding and public awareness initiatives. Community involvement is also critical in ensuring that these solutions are accessible and effective in mosquito-prone areas. Plant-based innovations, combined with strategic policy support and local action, can lead to a more sustainable approach to vector control, protecting public health and the environment for future generations.

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