



# A Review On Mosquito Repellent Action Of Nirgundi (Vitex Negundo)

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## ABSTRACT:

Vectors are one of the indirect modes of disease transmission which accounts for 17% of infectious disease causing 7 lakh death annually. Among vectors mosquitoes constitutes the most important single family of insects from the stand point of human health. They are found all over the world. The four important groups of mosquitoes in India which are related to diseases transmission are Anopheles, Culex, Aedes, and Mansonia. Commercial mosquito repellents contain N, N-diethyl-3-methylbenzamide (DEET) which has shown strong protection from mosquitoes but exerts toxic reaction under some circumstances in different age groups, hence alternative new products need to be explored. Vitex negundo, commonly known as the five-leaved chaste tree or "Nirgundi" in Ayurveda, is a medicinal plant widely known for its insecticidal and mosquito-repellent properties. Its ability to repel mosquitoes is attributed to its bioactive compounds, which act as natural repellents.

Key words: Mosquito, Repellent, *Vitex negundo*

## INTRODUCTION:

The mosquito poses a significant global health concern, serving as the primary carrier of numerous diseases impacting both humans and other animals. Members of the family Culicidae, within order Diptera, of the class Insecta, mosquitoes are identifiable by their slender physique, elongated legs, proboscis, and the presence of scales on various body regions. The species within these three genera serve as either primary or secondary vectors for deadly diseases such as malaria, dengue fever, yellow fever, filariasis, Japanese encephalitis, haemorrhagic fever, and chikungunya.

Mosquitoes utilize thermal, visual, and olfactory cues to locate a host, with olfactory stimuli being the most significant. Wearing dark-coloured clothing may also attract mosquitoes. Olfactory stimuli are especially crucial as mosquitoes approach the host, while visual cues aid in in-flight orientation. Two key chemicals released by the human body, carbon dioxide and lactic acid, serve as mosquito attractants. Carbon dioxide is emitted through exhalation and skin, attracting mosquitoes from distances of up to 36 meters. Lactic acid

stimulates the mosquito's chemoreceptors while mosquito repellents inhibit them, both located on the mosquitoes' antennae. Factors such as body temperature and moisture play important roles at close range. Additionally, scents from soaps, lotions, and perfumes may also act as attractants for mosquitoes.

Given the significant impact of mosquitoes on public health, various approaches have been employed to combat mosquito-borne infections. These methods include disrupting disease transmission by killing mosquitoes, preventing mosquito-human contact through repellents, and altering mosquito life cycles at different developmental stages. Mosquito management strategies aim to control mosquito populations effectively and efficiently while minimizing disruption to the ecological balance. These control measures can be biological, physical, genetic, or chemical in nature. Biological control involves utilizing natural predators of mosquito larvae and pupae, such as fish, birds, tadpoles, flatworms, and nematodes, to reduce mosquito populations. Physical methods of control include eliminating standing water sources where adult mosquitoes breed by filling ditches, drains, and covering water bodies. Genetic control methods involve breeding sterile male mosquitoes through radiation to reduce reproduction rates. Chemical control methods aim to eliminate mosquito larvae and adults using larvicides and adulticides, respectively. Moreover, the chemical-based mosquito repellents cause problems like skin irritation, erythema, desquamation and bullae formation on the skin of a person. DEET is most commonly used chemical mosquito repellent but it causes toxic effects like encephalopathy in children, anaphylaxis, urticaria syndrome etc.

The exploration of plant-based phytochemicals with repellent properties against vector mosquitoes has primarily been driven by the limitations associated with major chemical repellents like DEET and DDT, which suffer from issues related to resistance and toxicity. Phytochemicals derived from plants shows a potential substitute for synthetic insecticides or as complementary agents within integrated vector control programs. Phytochemical research has also demonstrated that traditionally used medicinal plants produce a diverse array of compounds with established therapeutic properties. Neem is widely recognized as a plant with antifeedant properties in certain regions. Various compounds extracted from *Azadirachta indica* have been identified as effective agents against mosquitoes.

Thus, various plant-based products renowned for their repellent and insecticidal properties are employed in mosquito control efforts. Presently, there is a growing interest in plant-based products due to the development of resistance and cross-resistance to chemical insecticides, along with increasing toxicity concerns and costs associated with their use. Furthermore, phytochemicals derived from the vast array of plant species are considered safe and biodegradable, making them suitable candidates for evaluation regarding their insecticidal and mosquito repellent capabilities, while also undergoing assessment for potential mammalian toxicity.

## GENERAL DESCRIPTION OF MOSQUITOES:

Mosquitoes (or mosquitos) are approximately 3,500 species of small flies comprising the family Culicidae (from the Latin *culex* meaning "gnat")<sup>1</sup>. The word "mosquito" (formed by *mosca* and *ito*) is Spanish for "little fly"<sup>2</sup>. Mosquitoes constitute the most important single family of insects from the standpoint of human health. The mosquitoes are at the centre of worldwide entomological research primarily because of their medical importance as vectors of dangerous diseases, such as Malaria, Yellow, Dengue, Zika, West Nile, chikungunya fever and other encephalitis, including Japanese encephalitis and lymphatic filariasis. More than half of the world's population lives under the risk of becoming infected by mosquitoes that carry the causative agents of these diseases. Estimates made by the World Health Organization (WHO) show that 247 million people became ill in 2006 and about 1 million people died (WHO 2008). Despite all efforts and significant reduction of malaria cases in 2018 still 228 million malaria cases occurred world-wide with more than 400,000 deaths (94% in Africa) (WHO 2019).

Mosquitoes are slender-bodied insects, typically measuring 3 to 6 millimetres in length. Their anatomy is divided into three main parts: the head, thorax, and abdomen. The head houses two compound eyes, antennae that help detect odors, and a specialized mouthpart called the proboscis. The proboscis is a long, tube-like structure used by female mosquitoes to pierce the skin of hosts and feed on blood, which is essential for egg production. The thorax supports a pair of scaled wings and three pairs of long, slender legs, giving mosquitoes their characteristic light and agile flight. Their abdomen is elongated and flexible, capable of expanding to store a blood meal. Most mosquitoes are grey, brown, or black, often with distinct patterns or stripes on their body or legs<sup>3</sup>.

Mosquitoes undergo a complete metamorphosis, consisting of four stages:

1. **Egg:** Female mosquitoes lay their eggs in stagnant water or moist environments. Some species lay their eggs in clusters called rafts, while others lay them individually.
2. **Larva:** Commonly known as "wigglers," mosquito larvae are aquatic and feed on organic debris in water. They breathe through a siphon and remain highly active.
3. **Pupa:** The pupal stage, also aquatic, is a non-feeding, resting phase where the mosquito develops into an adult.
4. **Adult:** After emerging from the pupa, the adult mosquito is capable of flight and begins its search for food.

The complete life cycle can range from 7 to 14 days, depending on environmental conditions such as temperature and humidity.

Mosquitoes are most commonly found in warm, humid climates, as they require water for breeding. Stagnant water bodies such as ponds, marshes, and even household containers provide ideal breeding grounds. While male mosquitoes feed exclusively on nectar, female mosquitoes require blood meals to obtain the protein necessary for egg development. They are attracted to hosts by detecting carbon dioxide, body heat, and specific odors.

Mosquitoes are most active during dawn, dusk, or nighttime, depending on the species. Certain species, such as *Aedes aegypti*, are active during the day, making them particularly dangerous vectors for diseases.

Mosquitoes are notorious for their role in transmitting diseases, making them one of the deadliest insects on Earth. Some of the major mosquito-borne diseases includes, Malaria which is caused by *Plasmodium* parasites and transmitted by *Anopheles* mosquitoes. Dengue Fever whose spread is by *Aedes aegypti* mosquitoes and caused by the dengue virus. Zika Virus which is known for its severe effects on pregnant women and their unborn children. Chikungunya, Yellow Fever, and Filariasis are also transmitted by different mosquito species. These diseases pose significant challenges to public health, particularly in tropical and subtropical regions.

In Ayurveda, we get the reference of *Mashaka*, which can be correlated with mosquitoes. The word *Mashaka* is derived from root *Mash*, *Ach prathyaya* and *Kan prathyaya*. It is a *Keeta visha*. "*Mashati dhwanati eti mashaka*"; that is, flies making sound<sup>4</sup>. It is having synonyms like *Vajra tunda* (the creature which has sharp features on its face), *oochyasya* (bears a pointed needle on its face) *Sookshma makshika* (has the size of a small honey bee) and *Rathri jagarada* (one which moves at night)<sup>5</sup>. There are 5 types of *Mashaka* according to *Sushruta*<sup>6</sup> which includes *Saamudra*, *Parimandala*, *Hasti mashaka*, *Krishna*, *Paarvatiya*. According to Kriya Kaumudi, 5 types are mentioned and not named individually<sup>7</sup>. In order to avoid the heat from the sun during the day, mosquitoes typically rest in the dark areas like homes, barns

for livestock, mixed use buildings etc, it is also said that they live in leather<sup>8</sup>. *Kandu, Shotha and Mandala* are the *Mashaka damsa lakshanas*<sup>9</sup>. Many *Dhoopana yogas* are mentioned in Ayurveda for mosquito repellency.

## MOSQUITO REPELLENT ACTION OF *Nirgundi*:

Plants have been used since ancient times to repel or kill blood-sucking insects in the human history and, even now, in many parts of the world people are practicing plant substances to repel or kill the mosquitoes. *Vitex negundo*, is a plant well documented medicinal properties including a potent larvicide. It possesses bioactive compounds that exhibit significant larvicidal activity against various species of mosquitoes. The larvicidal properties of *Vitex negundo* are attributed to the presence of several phytochemicals including flavonoids, alkaloids and essential oils like caryophyllene and sabinene. The leaf extracts of the plant with organic solvents have shown strong larvicidal effects, often leading to high mortality rates among larvae within 24-48hrs of exposure<sup>10</sup>.

The terpenoids and sterols in *Nirgundi* plays crucial role in mosquito repellent action. *Nirgundi* is having *Katu, Tikta, Kashaya rasa, Laghu, Ruksha guna* and *Ushna veerya*. It is mainly mentioned as *Kushtagna, Kandugna* and *Krimigna* by almost all authors. The bio active components are primarily found in the essential oils and extracts. Terpenoids are commonly found in the essential oils of *Nirgundi*.

Some important terpenoids found in *Nirgundi* include caryophyllene, sabinene, cineole and limonene. These compounds are volatile and emit strong fragrances that mosquitoes find repulsive. These compounds work by masking human odours such as carbon dioxide, lactic acid that attracts the mosquitoes, thus preventing them from detecting potential host. They also act as olfactory inhibitors, confusing the mosquito's sense of smell. Some of the terpenoids like cineole, have irritant properties for mosquitoes, making them reluctant to land or feed in areas treated with these compounds, also interferes with odorant binding proteins (OBP), disrupting mosquito olfaction and also modulates the GABA receptor activity thereby regulating mosquito neural excitability<sup>74</sup>. These terpenoid compounds like Vitexilactone, bind to octopamine receptors, essential for mosquito flight, feeding and reproduction. This blocks the receptors, disrupting the mosquito behaviour. Terpenoids like rotundifuran interacts with mosquito antennal receptors, responsible for detecting host cues (eg. lactic acid, carbon dioxide etc) and reduces mosquito attraction. Some terpenoids like negundilin inhibits Acetylcholinesterase (AChE) activity, an enzyme crucial for neurotransmission. This disruption affects mosquito nervous system function, leading to repellency. Some of the terpenoids like 5-hydroxy-3,6,7,4'-tetramethoxyflavone interacts with GABA receptors causing allosteric modulation where the compound binds to orthosteric sites on GABA receptors, altering receptor conformation and affecting GABA binding. It also enhances the GABA receptor activity, increasing chloride ion influx and hyperpolarizing neurons. The prolonged terpenoid exposure leads to GABA receptor desensitization, reducing neural activity. This effect on GABA receptors lead to reduced attraction, impaired flight and decreased feeding<sup>11</sup>.

The terpenoids interact with the GABA receptors, specifically GABA A and GABA B subtypes. It causes the allosteric modulation, which refers to the interaction between a molecule (e.g., terpenoids) and a receptor (GABA), enhancing or inhibiting its activity without binding to the primary binding site. The modulator binds to a secondary binding site, distinct from the primary GABA binding site. This binding induces a conformational change in the receptor, altering its activity. These allosteric modulators can either increase (positive allosteric modulator) or decrease (negative allosteric modulators) GABA receptor activity. The positive allosteric modulation enhances the GABA receptor activity, increasing the chloride ion influx and causes hyperpolarization. The negative allosteric modulation inhibits GABA receptor activity, reducing the chloride ion influx and depolarization. In silent allosteric modulation, it binds without affecting the basal activity but modifies receptor response to GABA. This disrupts the neural signalling,

affecting feeding, flight and survival. Also altered GABA receptor activity diminishes mosquito behaviour. Studies have shown that in *Nirgundi* a terpenoid Vitexilactone enhances GABA activity i.e., it is a positive allosteric modulator, another terpenoid named Rotundifuran inhibits GABA receptor desensitisation i.e., it is a negative allosteric modulator and another terpenoid named Negundilin allosterically modulates GABA receptors i.e., it is either negative or positive allosteric modulator<sup>12</sup>.

The chloride ion influx, triggered by the GABA receptor activation, contributes to mosquito repellency through Hyperpolarization, where there is an increased chloride ions which enters the neurons by hyperpolarizing the membranes. This hyperpolarization reduces neural excitability, disrupting normal signalling. Altered neural function affects the sensory processing, including olfaction. This reduced olfactory processing diminishes attraction to hosts. The effect of chloride ion influx on mosquitoes includes repellency, where the mosquitoes are repelled due to impaired sensory processing. Reduced attraction towards host causes impaired feeding behaviour. Also, neural disruption affects motor functions, impairing flight. Finally prolonged exposure leads to mortality<sup>13</sup>.

On the other hands sterols are a sub group of steroids. In plants they are known as phytosterols. Sterols found in *Nirgundi* include Stigmasterol, beta-sitosterol and campesterol. Sterols can contribute to the overall bioactivity of plant's extract, enhancing their mosquito repelling properties. They interfere with mosquito's cuticle (outer layer), affecting their ability to land or remain on the treated surfaces. Sterols creates pores or disrupts membrane integrity, leading to ion leakage, they can change the membrane's physical state, affecting protein function and also interfere with the signalling pathways thereby impairing mosquito responses. The beta -sitosterol interacts with phospholipid headgroups disrupting membrane structure and inhibits acetylcholinesterase which is essential for neurotransmission. Stigmasterol when inserted into the membrane alters fluidity and disrupts odorant-binding proteins impairing olfaction. Campesterol interacts with membrane bound receptors, modulating signalling and alters membrane potential by disrupting ion balance. Some studies suggests that sterols have anti-feeding properties, disrupting the feeding behaviour of the mosquito<sup>14</sup>.

Sterols from *Nirgundi* interfere with mosquito physiology through multiple mechanisms. It causes membrane disruption by interacting with membrane lipids, thereby altering the fluidity. The mechanism involved includes membrane fluidization, where sterols interact with cuticle lipids, increasing the fluidity. Sterols disrupts the lipid bilayer structure, compromising membrane integrity, they induce cholesterol efflux from cuticle membrane. The sterols modify the phospholipid composition, affecting membrane function. The effects on the cuticle of mosquito includes compromised membrane integrity enhances water and ion loss. Changes in the lipid composition affects membrane fluidity. The increased water loss disrupts the cuticle hydration balance and the compromised cuticle integrity reduces the mechanical strength. The stigmasterol disrupts the membrane fluidity and structure. Beta- sitosterol interferes with the cuticle lipids and cholesterol and Campesterol affects the phospholipid composition and membrane function. The physiological consequences on mosquitoes include dehydration, ion imbalance causing disrupted osmoregulation which affects the neural and muscular functions, impaired flight and reduced feeding behaviour<sup>15</sup>.

The terpenoids found in *Nirgundi* masks human odours through the following mechanisms. The terpenoids binds to mosquito olfactory receptors, blocking human odour detection. It competes with human odorants for receptor binding sites. They alter the receptor conformation, reducing the affinity for human odorants. Terpenoids accelerate odorant breakdown, reducing detection. The effect on mosquitoes includes reduced host attraction, impaired host detection and increased repellency. Vitexilactone is a potent olfactory receptor binder, rotundifuran is an allosteric modulator of olfactory receptors and negundilin is an odorant degradation accelerator.

Sterols from *Nirgundi* inhibits acetylcholinesterase in mosquitoes through mechanisms like competitive inhibition i.e., sterols compete with acetylcholine for AChE binding sites. Sterols binds to secondary sites, altering the AChE conformation and activity. Sterols interact with

AChE's hydrophobic regions, disrupting enzyme function. It also forms hydrogen bonds with the AChE residues, inhibiting substrate binding. The effect on mosquitoes includes neurotransmission inhibition

affecting the neural signalling, muscle contraction impairment affecting muscle function and movement, feeding behaviour disruption and increased mortality. The stigmaterol is a potent AChE inhibitor. Beta-sitosterol is an allosteric modulator of AChE and Campesterol causes hydrophobic interactions with AChE.

Sterols especially campesterol, disrupts the odorant binding proteins in the mosquitoes through hydrophobic interactions by altering protein confirmation. Campesterol forms hydrogen bonds with the odorant binding protein residues, disrupting the odorant binding. It creates allosteric modulations by binding to the secondary sites, altering the odorant binding protein's binding affinity for odorants. It also induces conformational changes in odorant binding proteins, affecting odorant recognition. The effect on mosquitoes includes impaired host detection, altered feeding behaviour, reduces mating due to disruption in pheromone detection and also prolonged exposure leads to increased mortality.

Thus, terpenoids acts as primary repellents, masking human odours and confusing mosquitoes with strong, volatile compounds while sterols contribute to the long-term effectiveness by potentially disrupting feeding behaviour and acting on mosquito's cuticle.

## CONCLUSION:

*Nirgundi* (*Vitex negundo*) is an effective, natural mosquito repellent with minimal environmental and health risks. Its bioactive compounds provide a sustainable solution to controlling mosquito populations and reducing the risk of mosquito-borne diseases. By incorporating *Nirgundi* in various formulations, both traditional and modern, we can harness its potential to create safer and more eco-friendly mosquito management strategies.

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