Study on mosquito repellent activities of *Vitex negundo* and its herbal computer incense bar

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(20PBC021)

Under the guidance of

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A Thesis submitted in

Partial fulfilment of the

Degree of Master of Science in Biochemistry

Avinashilingam Institute for Home Science and Higher Education for Women

Coimbatore – 641043

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CERTIFICATE

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INTRODUCTION

1. INTROCUCTION

Many of the vectors borne disease that harms humans and other animals are transmitted by mosquitos. Mosquitoes constitute a major public health problem as vectors of serious human disease. Hubalek and Haluzka reported that *Culex pipiens* is the vector of west Nile virus which cause encephalitis or meningitis which is known to affect the brain tissue, finally resulting in permanent neurological damage. There are several mosquito species that belong to the genera Anopheles, *Culex spp.* and *Aedes spp.* are vectors for disease such as malaria, filariasis, dengue fever, haemorrhagic fever and yellow fever (Mohan *et al*., 2007).

Mosquitoes are the most important arthropod vectors of serious human disease in terms of medicine. They spread malaria- causing protozoan parasites and viruses such as dengue fever, yellow fever, filariasis, chikungunya, Japanese encephalitis and others. Mosquitoes have recently been discovered to spread the zika virus, which the World Health Organisation (WHO) has designated a public health emergency of international concern. Every year, mosquito- borne disease incapacitate and severely disable millions of people, claiming countless lives. As a result, mosquitoes are regarded the world's deadliest animals, even more so than humans(Baden *et al*., 2016).

Malaria is an infection protozoan infectious. The pathogen is one of five plasmodium species that can cause disease in humans. Anopheles is the most common mosquito vector. Dengue fever is the viral infection. The pathogen is dengue virus. *Aedes spp.* is the most common mosquito vector. Acute febrile sickness can be caused by either disease. Malaria, on the other hand, can be chronic, whereas dengue cannot. The triads of dengue are atypical lymphocytosis, hemoconcentration and thrombocytopenia may be useful in distinguishing dengue infections from other tropical infection such as malaria. Every aliment has its own vector. The mosquito vector for each virus however is distinct habitat. The major habitat of malaria mosquito vector is in the forest, while the principal habitat of the dengue mosquito vector is in the city (Wiwanitkit, 2011).

The word repellent derives from Latin word meaning "to reject". Repellents can be dividing into five groups. 1. True repellent – mosquitoes are pushed away from an odour source without direct contact, 2. Contact irritants – it cause insect to orient away from a source after direct contact, 3. Deterrent – that inhibits a specific behaviour such as blood feeding, 4. Odour makers – that block attraction to humans, 5. Visual makers – prevent the localization of the host by an insect (Deletre *et al*., 2016).

DEET (N, N- diethyl -3- methylbenzamide) is the most common and efficient broad-spectrum insect repellent. It works for mosquitoes, ticks, biting flies, chiggers, and fleas and lasts for hours. The standard concentration of DEET in commercial products is 20-25 percent. The shorter protection time was also determined by the combination. DEET has a minor impact on the safety of commercial mosquito repellents. Disease control because to the high expense, disagreeable odour, and difficulty of continual application at high concentration on exposed skin(Murugan *et al*., 2019).

Indalone was found to be more effective than DEET at repelling mosquitos and ticks. However, in some test, indalone was found to be ineffective. Because of its low volatility, indalone is categorised as a gustatory or contact repellent, which means that the insect must make touch with the treated surface to be repelled. It seems to be safe for use on garments as well as direct skin application. Although indalone has a modest oral toxicity evidence of kidney and liver damage in rodents after prolonged exposure has limited its use(Ostfeld *et al*., 2006).

DMP, also known as dimethyl 1,2- benzenedicarboxylate, was one of the first synthetic repellent to be found, and was designed as a solvent in which numerous solid repellents were tested. Before being supplanted by newer active chemicals, DMP was widely used as board range repellent. It was widely used in china before being supersede by Quwenling, and it was the standard in India prior to the introduction of DEPA. DMP required to prevent mosquito bites was determined to be 8 – 8.15 mg per square inch. The repellent DMP is no longer in use (Gao and Wen, 2016).

The pyrethroid pesticide permethrin is derived from the plant *chrysanthemum cinerariifolium*. It was approved as an insect repellent and pesticide in the United States in 1979. It has recently become the most popular insecticide for usage on fabrics such as garments, bed nets, and other similar items due to its sole position as a contact insecticide via neural toxicity and as insect repellent. Permethrin- treated clothing is an important arthropod protection against a wide range of bloodsucking arthropods whileposing few safety risks, especially when used in conjunction with other protection strategies such as applying topical repellents(Vaz et al., 2019).

Nature is an endless source of inspiration for both individuals advancements. Natural repellents come from the Asteraceae, Cupressaceae, Labitae, Lamiaceae , Myrtaceae, piperaceae, Rutaceae and Zingiberaceae families. They have been tested for mosquito

repellency against a variety of vectors, but only a few been commercialised. The hydroxyl group is connected to a primary, secondary or aromatic carbon in the majority of arthropod-repellent compounds.

The repellent activity of several metabolites with hydroxyl group connected to a tertiary carbon, such as linalool, terpineol, and the limonene is inhibited against gambiae, implying that the kind of carbon where the hydroxyl substitution is present influences repellency(Khater *et al.,* 2016).

Around 1858, the term "Citronella" was created from the French word "citronelle". It was first extracted for use in perfumery and later employes to repel mosquitoes by the Indian army at the turn at the 20thcentury, before being registered for commercial usage in the United States in 1948. Citronella contains citrnellal, citronellol, geraniol, citral, pinene and limonene, which have comparable effect to DEET however the oils evaporate quickly, causing efficacy to be lost and leaving the user vulnerable. Citriodiol containing products had the mosquito repellent profiling among plant- derived compounds. Preventing mosquito bites may give crucial disease vector [rotection. Even though citronella- based repellents only provide protection from host- seeking mosquitoes for a limited period of two hours new formulations may be able to extend that duration(Murugan *et al*., 2016).

Neem offered 98.2 % protection against An. Darling for 8 hours. Despite the fact that it is not recognised by the US EPA for use as a topical insect repellent, neem is extensively promoted as a natural alternative to DEETand it has been evaluated for repellency against a variety of medical and veterinary arthropods. Bloodsucking mosquitoes, tabanids, ceratopogonids, simuliids, and licking flies were all repelled by a neem seed extract(Murugan *et al*., 2016)**.**

Methyl jasmonate which is generated from the non-volatile jasmonic acid has a much higher ultimate vapour pressur than DEET. It repels only Quinquefasciatus mosquitoes and not *Aede aegypti*, *Anopheles gambiae*, phelbotomus flies, limiting its use to Quinquefasciatus mosquitoes. Aversion to Methyl jasmonate has been seen in variety of ticks, including nymphal I. ricinis and Hyalomma marginatum rufipes among others (Galal *et al.,* 2016).

The plant material of the genus vitex is of prolific occurrence and about 15 species of them have been chemically examined. These species produce a wide range of metabolites which include mono, sesqui, di and triterpenoids, flavonoids, steroids, iridoids and their

glycosides, ecdysteriods, liganas, alkaloids, anthocyanidins and aromatic compounds. The volatile oil from the leaves has shown mosquito repellent activity. The leaf extract possesses an antiarthritic effect and smoke of its leaves is used to get relief from headaches(Amancharla *et al.*, 1999).

In search of new mosquito repellents from Indian plants, we examined the leaves of *Vitex negundo*which is known to have potential for pest control and insect repellency. In the present study we report making of computer incense bar from *Vitex negundo* and other 34 herbal products for the first time.

The present study was formulated with various tests with the following objective:

- To prepare the aqueous extract, ethanolic extract, chloroform extract and ethyl acetate extract with*Vitex negundo* leaves and checking their larvicidal activity on *Aedes spp.* and *Culex spp.*
- To prepare computer incense bar with herbal products, herbal products with panchakavyam and herbal products, panchakavyam along with *Vitex negundo* leaves.
- To check the mosquito repellency of computer incense bar prepared using herbal products, herbal products with panchakavyam and herbal products, panchakavyam along with *Vitex negundo* leaves against the mosquito species of *Aedes* and *Culex.*
    1. Cage test
    2. Excito- chamber
- To compare the *Vitex negundo*computer incense bar with commercially available mosquito repellent smoke coil using cage test.

The literature collection relevant to this study was done and the review of literature is presented in the following chapter.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. MOSQUITO BORNE DISEASE:

Mosquitoes, which belong to the phylum Arthropoda and the class Insacta, can transmit a wide range of viruses and parasites, including Malaria, Dengue fever, Chikungunya, Zika, Japanese encephalitis, and lymphatic filariasis, all of which are serious public health concerns.Drug prophylaxis and vector control are the sole alternatives for malaria, but there is no specific treatment for dengue; thus prevention through vector control measures is the only solution(Sogan *et al*., 2021).

Mosquito- borne infections continue to be a serious public health issue in both humans and veterinary medicine. Lymphatic filariasis is a parasitic infection that affects at least120 million people in 73 countries across Africa, India and the Middle East Islands in the Pacific Ocean. Not only they increase morbidity and death, but they also cost a lot of money and social upheaval in developing countries like India. India alone accounts for around 40% of global GDP. The burdenof filariasis and the anticipated annual economic loss is around 720 corers(Bagavan*et al*.,2011).

2.1.1. CLIMATE CHANGE, HEALTH AND MOSQUITO-BORNE DISEASE:

Dengue fever, chikungunya, Zika, Lymphatic Filariasis, Japanese encephalitis, Murray Valley, and others are among the many vectors - borne disease in the pacific, including those transmitted by mosquitoes. Malaria, Barmah forest virus illness, and Ross river fever and those diseases are particularly common in tropical areas, where the combination of a warm and humid atmosphere makes them more contagious. It provides perfect breeding circumstances for a variety of disease-carrying mosquito species. *Aedes spp.* mosquitoes are cold-blooded insects that need a certain temperature areas, as well as water reservoirs, in order to bread and develop. Development and life cycle of mosquitoes, and the pathogens they carry are highly dependent on local ecosystems.In the specific region, the *Aedes spp.* mosquito is a well-established vector of several viruses, primarily Zika (ZIKV), dengue (DENV), and chikungunya virus (CHIKV)(Filho *et al.*, 2019).

2.1.2 CHIKUNGUNYA:

Chikungunya is a mosquito-borne disease caused by the chikungunya virus (CHIKV), a member of the Togaviridae family of the genusAlphavirus. CHIKV produce a nonfatal,self-limiting disease marked by a sudden onset of high fever, acute arthralgia or arthritis, and a skin rash. In 1952-1953, HIKV was discovered after an outbreak in Tanganyika. The virus is said to have originated in Africa and spread to various parts of Asia after that. Thailand was the first Asian country to isolate CHIKV in 1958 followed by India in 1963. In Asia, Chikungunya is mostly occurring dengue - endemic areas and spread primarily by *Aedes aegypti* mosquitoes (Weaver *et al.,* 2015).

2.1.3 DENGUE :

Dengue fever is one of the most common mosquitos borne disease in the world. Dengue ranks as the most important mosquito borne viral disease in the world. In the past 50 years its incidence has increased 30-fold with significant out break occurring in five to six world health organisations(WHO). At, present, dengue is endemic in 112 countries in the world. Mosquitoes of the genus Aedes (*Aedes aegypti*, *Aedes albopictus*, *Aedes polynesiensis*) plays a critical role in the dengue fever transmission. *Aedes aegypti*, a day biting mosquito that breeds in containers, is found in tropical and subtropical climates. *Aedes aegypti* can reproduce in dirty water or in tiny groups such as flower vase or coconut shells, can be used to hold water(Malavige *et al.,* 2014).

2.1.4 MALARIA:

Malaria was almost eradicated in India in the early's 1960s, but it has since resurfaced as a major public health issue. The National Malaria Eradication Programme(NMEP) recorded 6.45 million cases in 1976. The impact was primarily on vivax malaria. Malaria which was once a rural illness, has evolved into numerous ecotypes as a result of development pressures. Forest malaria, urban malaria, industrial malaria have been recognised as ecotypes, with the latter spanning across epidemiological types (Wiwanitkit, 2011).

2.1.5 ZIKA :

ZIKA, an alphavirus, has resembles of CHIKV. Between the 1950s and 1980s, huge epidemics in India and Southeast Asia were caused by ZIKA. Which was first discovered in Africa in1952. ZIKV's adaption to an urban or peri-urban cycle, which involves *Aedes aegypti* and other mosquitoes of the stegomyia subgenus as vectors and humans as amplification hosts. The potential for massive urban epidemics of ZIKA,DENV, CHIKV, yellow fever, epidemic

polyarthritis are mosquito-borne virus exists because more than half of the world's human population lives in mosquito- infested areas (Musso *et al*., 2015).

2.2.1. COMMERICALLY AVAILABLE MOSQUITO REPELLANTS:

Repellent is derived from the latin verb repellere, which means "to refuse". By nullifying an insect attraction to an odour source, a repellent is a chemically volatile substance that leads a responder to aggressively travel in the opposite direction from the stimulus source(Debboun and strickman, 2013).

Repellents are classified in to five categories on the basis of observed insect behaviour: 1.True repellent – also called expellant, spatial repellent that push mosquitoes away from an odour source without direct contact. 2. Contact irritants – also called landing inhibition or excito repellent that cause insect to orient away from the source after direct contact. 3. Deterrent- also called as antifeeding, suppressant, anorexigenic and antiappetant is a substance that inhibits a specific behaviour such as blood feeding or oviposition. 4. Odour Maskers – also called attraction inhibition that blocks attraction to humans either by reducing the attractiveness of the host or an interruption of the localization of the host by the odour cue signal. 5. Visual makers – that disrupt visual cue and thereby prevent the localization of the host by an insect (Deletre *et al*., 2016).

2.2.2. Synthetic Repellents:

Dimethyl Phthalate (DMP):

DMP, also known as dimethyl 1,2-benzenedicarboxylate, was one of the first synthetic repellents to be found and it was originally designed as a solvent in which several solid repellent could be tested. However, DMP was less effective against mosquitoes, and the lowest dose of DMP required to prevent mosquito bites was determined to be 8 to 8.15 mg per square inch, compared to 0.36 to 0.50 mg per square inch for DEET (N,N- diethyl- m- toluamide). DMP is no longer utilised as a repellent, but it is widely employed as a solvent in the pharmaceutical sector to hold colour and aroma in a variety of consumer and personal care goods (Gao and Wen, 2016).

Figure: 1- Dimethyl Phthalate

Picture source:

https://www.cpiinternational.com/media/catalog/product/cache/image/300x300/e9c3970ab036de70892d86c6d221abfe/z/-/z-010039-02.jpg

INDALONE:

Indalone (butyl 3,4-dihydro-2,2 – dimethyl-4 –oxo-2H- pyran- 6- carboxylate) was found to be more effective than DEET at repelling mosquitos and ticks. In some test, indalone was found to be ineffective. Because of its low volatility, Indalone is categorised as a gustatory or contact repellent, which means that the insect make touch with the treated surface to be repelled. Although indalone has a modest oral toxicity evidence of kidney and liver damage in rodents after prolonged exposure (Carroll *et al*, 2010).

DEET:

DEET is an acronym of N,N- diethyl –Meta -toluamide which has been renamed as N,N-diethyl-3-methylbenzamide. Because DEET is the most widely used insect repellent, researches have concentrated their efforts on identifying insect olfactory receptors and neurons. The mosquito olfactory system detects DEET when it is applied to the skin system as aversive volatile cue from near proximity, but there are two competing hypotheses at the moment. 1. Activation of the ionotropic receptor Tr40a and 2. Activation of the ionotropic receptor Ir40b

olfactory receptor. DEET has a relatively restricted role die to its high cost and disagreeable odour and the inconvenient of continuous application on the exposed skin at high concentration(Deletre *et al.*, 2016).

Figure: 2 - N, N-diethyl-3-methylbenzamide (DEET)

Picture source:

https://encrypted-tbn0.gstatic.com/images?q=tbn:ANd9GcQPp_-CthCF1IgzHcHr99JikyDpSkQuPjB7lQ&usqp=CAU

PERMETHRIN:

Permethrin is a synthetic pyrethroid pesticide derived from the plant *Chrysanthemum cinerariifolium*. It is odourless and biodegradable. Permethrin works primarily through the neurological system of insect, inhibiting sodium ion entry into nerve cells by inhibition of acetylcholinesterae, adenosine triphosphatase, and the gamma aminobutyric acid-A receptor resulting in paralysis. Clothing coated with permethrin offers higher protection against *Aedes spp.* and Anopheles species but not against *Culex* Species. Permethrin poisoning is uncommon; but excessive application at high dose can cause neurotoxic symptoms such as tremors, loss of coordination, hyperactivity, paralysis and an increase in body temperature. Other side effects include skin and eye irritation, reproductive effects, mutagenicity and alteration in the immune system(Cisak *et al*., 2012).

Figure: 3 - Permethrin

Picture source:

https://i5.walmartimages.com/asr/d86ffdb0-af7d-4c64-9539-a81899cb8ca3_1.cd8716d34d2b4217a51bd8b0da94ba71.jpeg

PICARDIN:

Picardin is a colourless, odourless piperidine analogue developed by bayer utilising molecular modelling in the 1980s. Picaridin's efficacy is equivalent to DEET, and a 20% picaridin spray wsa found to provide against three mosquito vectors, namely *Aedes spp.*, Anopheles and *Culex spp.* for roughly 5 hours than DEET. However, every 4-6 hours, reapplication is required. The chemical has a low toxicity and is less irritating to the skin and olfactory system. Picaridin is also non-toxic to plastic and synthetics, unlike DEET. DEET's ease of use and low toxicity profile make it an appealing and unquestionable acceptable option for protection against a variety of hematophagous arthropods and the threat of vector-borne disease in endemic areas**(**Leal, 2014**).**

Figure: 4 - Picardin

Picture source:

https://m.media-amazon.com/images/I/61pWlohVyGL._SL1500_.jpg

2.3.Natural Repellent:

Nature has long served as a source of inspiration for both humans and scientific and technological advancements. Recently, there has been lot pH focus on the medical effects of plant extract all around the world. Natural repellents come from the Asteraceae, Labiatae, Meliaceae, Myrtaceae, Piperaceae, Umbelliferae and Zingiberaceae families. They have been tested for mosquito repellency against a variety of vectors, but only a few have been commercialised. After the United States Environmental Protection Agency (US EPA) in 1986 exempting substances determined to be minimum harmful pesticides, interest in plant-based insect repellents grew(Khateret al, 2019).

Some metabolites in essential oils, such as the monoterpenes- pinenee, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor and thymol are mosquito repellents. The seequeterpene caryophyllene ie repellent against *Aedes aegypti*, and phytol, a linear diterpene. The hydroxyl group is connected to primary, secondary or aromatic carbon in majority of arthropod repellent chemicals. The repellent activity of several metabolites with a hydroxyl group connected to a tertiary carbon, such as linalool, terpineol and limonene, is inhibited against A. gambiae, implying that the kind of carbon where the hydroxyl substitution is present influences repellency. Volatile terpenoids like terpinen-4-ol make up the majority of insect repellents (Vaz *et al*., 2019).

2.3.1. LEMON EUCALYPTUS:

Lemon eucalyptus or *corymbia citriodora* (Myrtaceae) is a powerful natural repellent derived from the leaves of lemon eucalyptus trees. Because of the fresh scent of lemon eucalyptus essential oil, which contains 85% citronellal, it was discovered that the waste distillates left after hydro-distillation of the essential oil was significantly more effective at repelling mosquitoes than the essential oil itself. Because their active components are highly volatile, they are effective repellents for only a brief time after application before quickly dissipating, leaving the person vulnerable (Maia *et al.,* 2011).

PMD from lemon eucalyptus extract:

In the 1960's, PMD (Para-methane 3-8, diol) was discovered to be effective and commercially accessible repellent. PMD is the only plant-based repellent that the centres for disease control (CDC) has recommended for use in disease- endemic areas because of its clinical efficacy in preventing malaria and the fact it poses no harm to human health. The Environmental Protection Agency (EPA) has not approved lemon eucalyptus essential oil for use an insect repellent (Moore *et al.,* 2011).

Figure: 5- Lemon eucalyptus

2.3.2. CITRONELLA:

Essential oils and extracts from plants in the genus Citronella are frequently utilised as ingredients in plant based insect repellents. It was first employed to repel mosquitoes by the Indian Army at the turn of 20th century, and it was later registered for commercial use in the United States in 1948. Encapsulated citronella oil nanoemulsionis made by homogenising 2.5% surfactant and 100% glycerol under high pressure to generate stable droplets that promote oil

retention and slow release. Because the release rate is inversely to the protection period, a reduction in the release rate might extend mosquito protection time. Microencapsulation with geletin - Arabic gum microcapsules, which kept citronella repellent for up to 30 days, is another way to extend the action of natural repellents(Moore *et al*., 2011).

Figure: 6 - Citronella

Picture source:

https://hips.hearstapps.com/hmg-prod.s3.amazonaws.com/images/citronella-mosquitoes-1558117473.png

2.3.3. NEEM :

Neem, *Azadirachta indica*, Carapa procera, Melia azadarach, Khaya senegalensis, and Trichiliaemetica are aromatic plants in the meliaceae family that contain limonoid compound that have insecticidal and repellent properties. Neem offered 98.2% protection against *Aedes spp.* for 8 hours. Despite the fact that the US EPA has not certified neem for use as a topical insect repellent, it was extensively promoted as a natural alternative to DEET. It has been tested for repellence against a wide range of arthropods of medical and veterinary importance. Mitestop based on a neem seed extract, had a considerable repellent effect on bloodsucking mosquitoes, tabanids, ceratopogonids, simuliids, as well as licking flies(Govindarajan *et al*., 2019)

Figure: 7 - Neem

2.3.4. Methyl jasmonate :

Methyl jasmonate is generated from the non-volatile jasmonic acid and has a vapour pressureof 0.001mm Hg at 25℃, which is significantly greater than DEET. It repels only quinquefasciatus mosquitoes and not *Aedes aegypti,Anopheles gambiae*, *Phlebotomus flies*, or *Glossina Morsitans*, limiting its use to Quinquefasciatus mosquitoes. In ticks such as nymphal I. ricinis and Hyalomma marginatum rufipes Koch on the other hand, methyl jasmonated has been demonstrated to cause aversion (Govindarajan *et al*., 2019).

2.4.1. Plant source:

Many plants have anti- insect repellents properties, which are necessary for disease protection. Because they are plant- based, they are environmentally friendly and have few adverse effects. Essential oils from one or more the following plants are found in several plants-based insect repellents now on the market: geranium, lemongrass, peppermint, cedar, geranium, eucalyptus neem, marigold and soybean. As a result, they are a powerful source of repellent action. They provide protection by doing so. It works against mosquitoes and no negative side effects. Because of its negative effects, synthetic mosquito coli can be harmful. Natural incense bar on the other hand are effective and valuable.

Vitex negundo:

TAXONOMIC CLASSIFICATION:

- Kingdom - Plantae
- Sub kingdom - Tracheobionta
- Super division - Spermatophyta
- Division - Magnoliophyta
- Class - Magnoliopsida
- Sub class - Asteridea
- Order - Lamilales
- Family - Verbenaceae
- Genus - Vitex linn
- Species - *Vitex negundo* Linn.

Figure: 8 – *Vitex negundo*

The plant material of the genus vitex is of prolific occurrence and about 15 species of them have been chemically examined. These species produce a wide range of metabolites which include mono, sesqui, di and triterpenoids, flavonoids, steroids, iridoids and their glycosides, ecdysteroids, lignans, alkaloids, nthocyanidins and aromatic compounds. The chemical constituentsand their biological activity of *Vitex negundo*were reviewed recently. Although the volatile oil from the leaves has shown mosquito repellent activity, no active principle has been isolated. The leaf extract possess an antiarthritic effect and smoke of its

leaves is used to get relief from headaches. In search of new mosquito repellents from Indian plants, we examined the leaves of *Vitex negundo*, which is known to have potential for pest control and insect repellency(Amancharla *et al.,* 2000).

Herbal pesticides have recently acquired popularity, plant products were far safer and more environmentally friend for pest control even before chemical pesticides were introduced. The management of insect pest, eco-friendly biological agents are used. In this attempt has been made to test the repellent and larvicidal properties of leaf extract of *Vitex negundo*(Govindarajan *et al*., 2008).

2.4.2. Selected herbs:

1. *Chryspogan zizanioides* (Vetiver):

Chryspogan zizanioides plant originated in India and its grass and roots contain essential oil that have been used extensively for perfumes, cosmetics, deodorants, lotions, soaps and aromatherapy application. On the analysis vetiver oil has been shown to contain more than 100 compounds, mainly sesquiterpenes and their derivatives. This oil is also shown to have insecticidal, antioxidant and anticancer activities (Sikka *et al*., 2018).

2.*Anisomeles Indica* (Peimirati):

Leaves juice is given orally to treat fever and stomach ache. The leaves juices is given internally to cure scorpion sting and snake bite. Common name in India is Indian catmint (Kannadhasan *et al*., 2016).

3.Brassica juncea (Venkadugu):

Brassica juncea is one of six cultivated Brassicaceae species, and it is major oil yielding crop. Brassica oil seed production represents 14%of the edible oil production, ranking third after palm and soybean edible oil. *Brassica juncea* is not a common disease, but it is used in folk medicine. The root is used as a galactagogue in Africa and its ingestion may impart abody odour that serve as mosquito repellent. It is used to treat skin condition(Velez, 2017***).***

4.*Nardostachys jatamansi* (Sada maanji):

It is found in Himalayan region and it is used as indigenous drugs. The roots and the rhizomes used in ayurvedha, have been used in various herbal formulation including dietary supplements. The sesquiterpenes, lignans and neolignansare reported to be present in the roots

of the plant. The decoction of the drug is also used in neurological disorders, insomnia and disorders of cardiovascular system(Pandey *et al.,* 2013*).*

5. *Hemidesmus indicus*(Nanari):

It is commonly known as Indian sarsaparilla is a diffusely twining undershrub having numerous slenser wiry lactiferous branches with purplish brown bark. The milky latex of the plant is used for relieving inflammation in the eye. Ether extract of the root exerts some inhibitory effect on the growth of *Escherichia coli*. Bacteriostatic, anticancer, antiviral, antilithic, hypotensive, antifungal, antibacterial, anti-inflammatory, spasmodic activities have been reported (George *et al*., 2008).

6. *Leucas aspera* (Dumbai):

The plant is used traditionally as an antipyretic and insecticide. Medicinally, it has been proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive and cytotoxic activity and as antidote to snake venom. Leucas leaves are used in chronic rheumatism. The presence of various phytochemical constituents mainly triterpenoids, oleanolic acid, urosolic acid and b-sitosterol, nicotine, sterols, glucoside, diterpenes and phenolic compounds(Srinivasan, 2011)

7. *Tinospora cordifolia* (Seendil):

It is a large, glabrous, perennial, deciduous tree found throughout India. It is widely used plant in folk and ayurvedic systems of medicine. Tinospora cordifolia has been used in Ayurvedic preperetion for the treatment of various ailments throughout the centuries. It is ued as a rasayana to improve the immune system and body resistance against infection. Due to higher alkaloid content in the stems than in the leaves.It is claimed to be useful in treating leprosy, fever, asthma, anorexia, jaundice, gout, skin infection, diabetes, chronic diarrhea (Upadhyay *et al*., 2010)

8. *Brophyllum pinnatum* (Navamara):

It is a perennial herb commonly known as patharcatta. It is widely distributed in Madagascar, tropical Africa, tropical America, India, China and Australia. In Ayurvedha, the plant is also known as pasanabheda which means dissolver of stones. Leaves are known to possess neurosedative and muscle relaxant, antimicrobial, antiulcer, uterine contractility and anti-inflammatory activities. The medicinal and pharmacological properties of *Bryphyllum*

pinnatum are ascribed to the presence of alkanes, alkanols, triterpenes and sterols, triterpenoids and phenanthrenes, glycosides and lipids (Mahendra Yadav *et al*., 2016)

9. *Aegle marmelos* (Vilva):

It commonly known as vilva or sriphal or shivadruma.These trees are indigenous to India and are foundgrowing in abundance in the Himalayan regions. Vilva is reported to contain biologically important phytochemicals such as the volatile compoundslimonene, β-phellandrene,linalool, cineole and citronella. The roots and barks of the tree are used in treating fever. The leaves have astringent, febrifuge and expectorant properties are useful in remedy of fevers. They are good for dropsy, bleeding flies, dysentery, diarrhea and bowel complaints (Baliga*et al*., 2013)

10. *Eclipta alba* (Karisalankanni):

Traditional medicinal system of Indian subcontinent countries as well as tribal practitioners consider the plant to have diverse medicinal values and use it commonly for treatment of gastrointestinal disorders, respiratory tract disorders, fever, hair loss and greying of hair, liver disorders. The plant has several phytoconstituents like wedelolactone, eclalbasaponins, ursolic acid, oleanolic acid, luteolin and apigenin. Phytoconstituents like wedelolactone and ursolic and oleanolic acids can from the basis of new drugs against cance. (Jahan *et al*., 2014).

11. *Cassia auriculata*(Avaram):

Cassia auriculata is a shrub with large bright yellow flowers which is distributed throughout hot deciduous forests of India and holds a very prestigious position in Ayurveda and Siddha systems of medicine.The plants contain preliminary phytochemical constituents such as alkaloids, phenols, glycosides, flavonoids, saponins, proteins, carbohydrates and anthraquinone derivatives and, these are responsible for the pharmacological activity (Aparna *et al* ., 2018).

2.5 ASSAYS:

2.5.1. Cage test:

The mosquitoes utilised in the experiment must be pathogen n- free. According to WHO guideline for efficacy repellents, the cage measurement should be between 35-40 cm on

each side. Some studied have documented a cage dimension. Bano et al employed a cage with diameter of 18x18x18 cm (*Bano et al., 2014)* while Phasomkusolsil et al employed dimension of cage 30x30x30 cm (*Phasomkusolsil and Soonwera 2011),* Anitha et al employed a 34x32x32 cm cage dimension, while Chang et al., 2006 reporteda cage size 35x35x35 cm.

The cage was fitted with transparent mosquito netting to allow for easy observation as well as protection. Keep the mosquitoes contained within the cage. It contains holes for incense bar access that are likewise covered with netting. The cage must be filled with 200 mosquitoes that have been deprived overnight and only fed sucrose solution according to WHO. Then the *Vitex negundo* computer incense bar were kept inside the cage and then the mosquitoes in the cage were exposed to the smoke of computer incense bar for 45 minutes and the mortality data were recorded after every 15 minutes

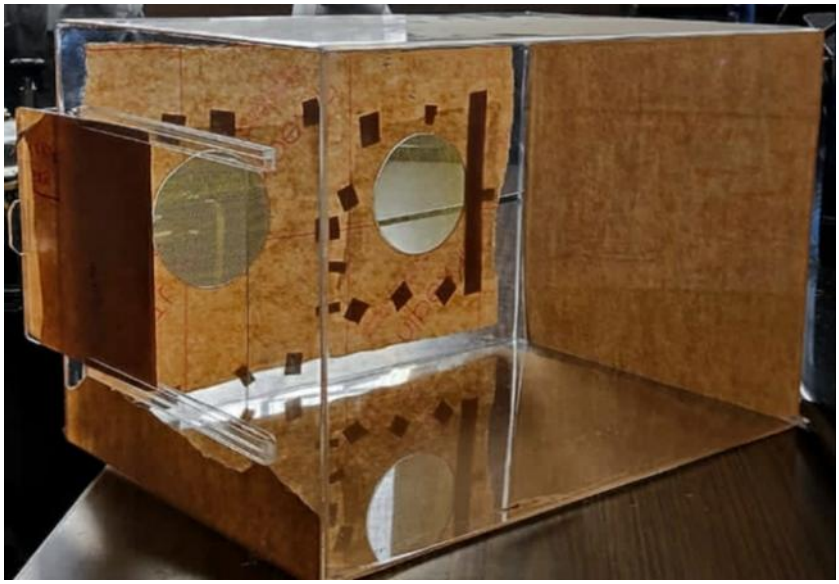


Figure: 9 – Cage Test

2.5.2. <u>EXCITO- CHAMBER TEST:</u>

The excito- chamber method is a modified custom method to observe the mosquito behaviour change in the form of moving away from area with incense bar to area without incense bar. This method and Cone test method does not involve the human subject to lure the mosquito. However, both methods can determine the behaviour of the mosquitoes towards the incense bar. The box is made with one front and exit panel occupied with single escape portal. It builds up with screened inner chamber, glass holding frame and door cover. The mosquito was starved overnight or least minimum 4 hours before the test. The behaviour of mosquito was

observed in term of number of escaped mosquitoes to another space and remains mosquitoes inside the chamber which filled with treated product. The observation is recorded after 10 and 30 min exposure. The test was conducted in daylight and repeated for four times. The percentage of mosquito repellencewas calculated using the formula(Adeela *et al*., 2016).

% Mosquito repellence = (NES + NDE) / (NEX) X 100

Where NES corresponds to the number of mosquitoes escaped, while the NDE refer to the number of mosquitoes dead and last is NEX represents the number of mosquitoes exposed.

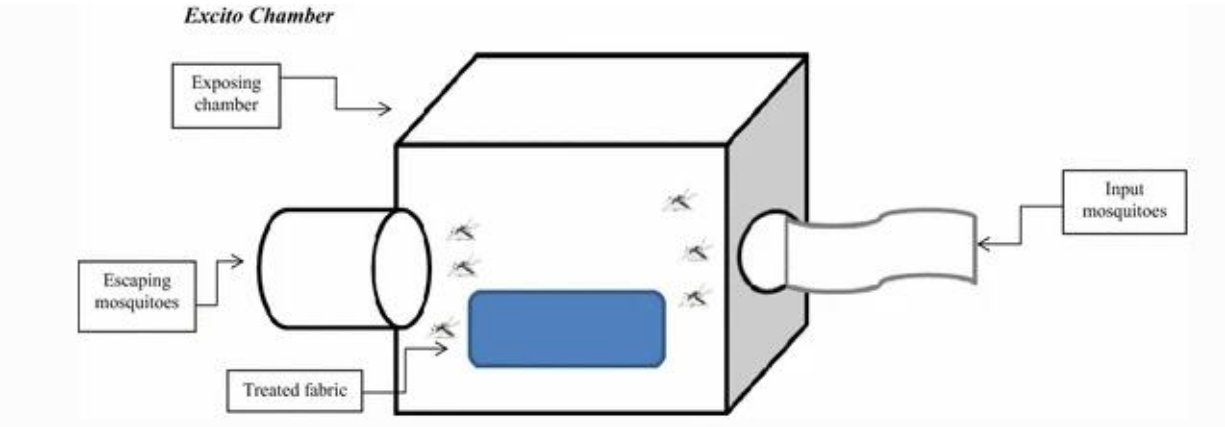


Figure: 10 – Excito chamber

Picture Source:

https://media.springernature.com/lw685/springerstatic/image/art%3A10.1186%2Fs40691-016-0064-y/MediaObjects/40691_2016_64_Fig3_HTML.gif

Many of the vectors borne disease that harms humans and other animals are transmitted by mosquitoes. Mosquitoes constitute a major health problem as vectors of serious health problems as vector of serious human disease. Immediate action is must to control the mortality and morbidity rate minimum; this can be done by insect repellents which are chemical or organic substance. Despite the fact that chemical insect repellent are safe, some side effects have been reported as result of improper use, this can be overcome by herbal insect repellent.

The procedure to prepare the herbal mosquito repellent and to check its repellence is given in the following chapter.

METHODS

The present study focused mainly on the mosquito repellent activity of the computer incense bar of *Vitex negundo* against *Aedes spp.* and *Culex spp.*

3.1 Plant collection:

Fully completed fresh leaves of *Vitex negundo* were collected from local area of Sathyamangalam, Erode. Leaves were washed with water and dried under shade at room temperature for 5-7 days and were powdered using mortar and pestle.

3.2 Mosquito and larva collection :

Mosquito larvae and pupae of *Aedes spp.* and *Culex spp.*were collected from Nation Centre for Disease and Health Control (NCDC), Mettupalayam. It was reared at room temperature around 25-27℃which is similar to the environment from which they are isolated. The larvae were reared in 500ml white plastic container. Net is used to covered the container so as to allow sufficient oxygen and light penetration. The larvae and pupae were kept in the water medium and fed with small quantities of baker's or brewer's yeast once in every two days so as to avoid fermentation and development of fungus. Larvae pupate within 3-4days and pupae emerges as adult in a day or two under favourable condition. Immediately the adult emerges they leave the water surface and attach themselves to the walls of the container and net. The container was placed in the rearing cage and the net was removed carefully so as to transfer all the emerged adult mosquitoes into the cage(Umar *et al*., 2021).

3.3 Larvicidal Activity:

[A]. Preparation of Aqueousextracts:

The plant sample was collected from nearby garden in Sathyamangalam. 5g of fresh samples were collected,cleaned, grinded and extracted using distilled water. The extract was evaporated to dryness at 60℃and concentrations ranging from 20mg were dissolved in 5µl of dimethyl sulfoxide.

[B]. Preparation of plant extract:

The plant sample was collected from nearby garden in Sathyamangalam. Five grams of fresh samples were collected, cleaned, grinded and extracted using ethanol. The extract was evaporated to dryness at 60℃and concentrations ranging from 20µgwere dissolved in

5µl of dimethyl sulfoxide. Similarly, the plant extract was prepared by using ethyl acetate and chloroform.

3.3.1. Biological assay:

Twodifferent concentrations of (20µg/ml and 40µg/ml) aqueous extract, ethanolic extract, chloroform extract and ethyl acetate extract were prepared and checked for their larvicidal activity. All experimental exposures were made in petri- plate. Fifteen (15) larvae were collected with a Pasteur pipette and placed in a petri- plate containing two different test samples. Control test was performed with distilled water only. The petri- plate were covered with muslin cloth to avoid entry of any foreign material. The observed mortality was recorded at 24 hours of exposure to test solution. From this data with respect to mortality, larvicidal activity of *Vitex negundo* and the percentage mortality was recorded.

$$\text{Percentage mortality} = \frac{C - T}{C} \times 100$$

C = No. of larvae survived in the control; T = No. of larvae survived in the test sample

3.4. Preparation of incense bar:

3.4.1. Preparation of mosquito incense bar paste:

[A]. Herbal base incense bar preparation:

Powdered forms of *chrysopogon zizanioides* (vetiver), *Indian catmin* (peimiratti),*White mustard* (venkadugu), *Erucastrum gallicum* (naaikadugu), seeds of *Lawsonia innermis (*henna), *Nardostachys jatamansi* (jadamanji), *Senna auriculta* (aavarm poo), *Hemisdesmus indicus* (nannari), *Helicteres isora* (Valampuri kaai), *Cyperus rotundus*(korai kilangu), *Azadirachta indica* (neem), barks of *Ficus religiosa*(arasanguchi),barks of *Ficus benghalensis* (bannian),barks of *cedrus deodara* (devadara pattai),barks of *Acacia catchu* (karungali pattai), *Syzygium jambolanum* (Indian black berry),*Erythroxylum indicum* (agil),*Wrightia tinctoria* (thugil), *Ocimum tenuiflorum* (thulasi), *Aegle marmelos* (vilvam), *Leucas aspera* (thumbai), *Tinospora cordifolia* (seendhal), *Eclipta prostrate* (karisalanganni), *Solanum procumbens* (thudhuvalai), *Cynodon dactylon* (arugampul), *Cinnamomum camphora* (camphor), *Shorea robusta*(kungliyam) were mixed together to make herbal base incense bar.

[B].Panchakavyam and herbal base incense bar preparation:

Panchakavyam is a mixture used in traditional Hindu rituals that is prepared by mixing five ingredients. The three direct constituents are cow dung, urine, and milk; the derived products are curd and ghee. And jaggery is used as binding agent and mix with herbal base for making computerincense bar.

[C].*Vitex negundo*, herbal base and panchakavyam incense bar:

Vitex negundo leaves were collected from sathyamangalam. They were cut into small pieces and shade dried for about two days. The dried parts were pounded and powdered using domestic grinder and mixed with herbal base and panchakavyam for making computer incense bar.

3.4.2 Moulding of mosquito paste into incensebar:

The mould was formed with a length of 66 cm and width of 7 cm and height of 4 cm. Then the mosquito paste was slowly poured into mould and the paste was sundried for about 36 hours. The incense bar was removed from moulder.

Figure: 11 – Computer incense bar prepared

A. Using *Vitex negundo*leaf, herbal base and panchakavyam

B. Using Herbal base with panchakavyam

C. Using Herbal base

3.4.3Mosquito repellent Activity:

Cage test:

The cage was fitted with transparent mosquito netting to allow for easy observation as well as protection. Keep the mosquitoes contained within the cage. It contains holes for incense bar access that are likewise covered with netting. The cage must be filled with 20 mosquitoes that have been deprived overnight and only fed sucrose solution according to WHO. Then the *Vitex negundo* computer incense bar were kept inside the cage and then the mosquitoes in the cage were exposed to the smoke of computer incense bar for 45 minutes and the mortality data were recorded after every 15 minutes (Adeela *et al* ., 2016)

EXCITO- CHAMBER TEST:

The excito- chamber method is a modified custom method to observe the mosquito behaviour change in the form of moving away from area with incense bar to area without incense bar. However, excito- chamber methods can determine the behaviour of the mosquitoes towards the incense bar. The box is made with one front and exit panel occupied with single escape portal. It builds up with screened inner chamber, glass holding frame and door cover. The incense bar prepared with herbal base, panchakavyam and *Vitex negundo* leaves is placed inside the chamber. The mosquito was starved overnight or least minimum 4 hours before the test. The behaviour of mosquito was observed in term of number of escaped mosquitoes to another space and remains mosquitoes inside the chamber which filled with treated product. The observation is recorded after 10 and 30 min exposure. The test was conducted in daylight and repeated for four times. The percentage of mosquito repellence was calculated using the formula (Adeela *et al.*, 2016).

% Mosquito repellence = (NES + NDE) / (NEX) X 100

Where NES corresponds to the number of mosquitoes escaped, while the NDE refer to the number of mosquitoes dead and last is NEX represents the number of mosquitoes exposed.

3.4.4. Comparative studies:

Commercially available mosquito repellent, good night and maxo were checked for their repellence and compared with incense bar made with *Vitex negundo* by cage test

Cage test:

The cage was fitted with transparent mosquito netting to allow for easy observation as well as protection. Keep the mosquitoes contained within the cage. It contains holes for incense bar and commercially available mosquito coil access that are likewise covered with netting. The cage must be filled with 20 mosquitoes that have been deprived overnight and only fed sucrose solution according to WHO. Then the *Vitex negundo* computer incense bar were kept inside the cage and then the mosquitoes in the cage were exposed to the smoke of computer incense bar for 45 minutes and the mortality data were recorded after every 15 minutes (Adeela *et al* ., 2016).

RESULTS AND DISCUSSIONS

4. Result and Discussion

Mosquitoes are the most important arthropod vectors of serious human disease in terms of medicine. They spread malaria- causing protozoan parasites and viruses such as dengue fever, yellow fever, filariasis, chikungunya, Japanese encephalitis and others. Mosquitoes have recently been discovered to spread the zika virus, which the World Health Organisation (WHO) has designated a public health emergency of international concern. Every year, mosquito- borne diseases incapacitate and severely disable millions of people, claiming countless lives. As a result, mosquitoes are regarded the world's deadliest animals, even more so than humans (Baden *et al*., 2016).

Nature has long served as a source of inspiration for both humans and scientific and technological advancements. Natural repellents come from the Asteraceae, Labiatae, Meliaceae, Myrtaceae, Piperaceae, Umbelliferae and Zingiberaceae families. They have been tested for mosquito repellency against a variety of vectors, but only a few have been commercialised. After the United States Environmental Protection Agency (US EPA) in 1986 exempting substances determined to be minimum harmful pesticides, interest in plant-based insect repellents grew(Khateret *et al*., 2019).

The plant materials of the genus vitex are of prolific occurrence and about 15 species of them have been chemically examined. The chemical constituents and their biological activity of *Vitex negundo* was reviewed recently. Although the volatile oil from the leaves has shown mosquito repellent activity, no active principle has been isolated. In search of new mosquito repellents from Indian plants, we examined the leaves of *Vitex negundo*, which is known to have potential for pest control and insect repellency(Amancharla *et al.,* 2000).

The present study aimed to evaluate a larvicidal activityof aqueous extract, ethanolic extract, chloroform extract and ethyl acetate extract were prepared using *Vitex negundo*leaves and mosquito repellent activity of incense bar made by using *Vitex negundo* leaves and the result obtained are presented in this chapter.

4.1. Larvicidal activity:

Table:4.1.a - Larvicidal activity of aqueous, ethanol, chloroform, ethyl acetate extract of *Vitex negundo* leaves against *Aedes spp.*

Solvent Extract tested	Concentration (µg/ml)	No. of larvae		Time duration (Hours)	Percentage mortality
		Exposed	survived		
Distilled water (Control)	-	15	0	24	0
Aqueous Extract	20	15	11	24	26
	40	15	11	24	26
EthanolExtract	20	15	6	24	60
	40	15	5	24	66
ChloroformExtract	20	15	12	24	20
	40	15	9	24	40
Ethyl acetateExtract	20	15	13	24	13
	40	15	12	24	24

Figure 12 -Larvicidal activity of aqueous, ethanol, chloroform, ethyl acetate extract of *Vitex negundo* leavesagainst *Aedes spp.*

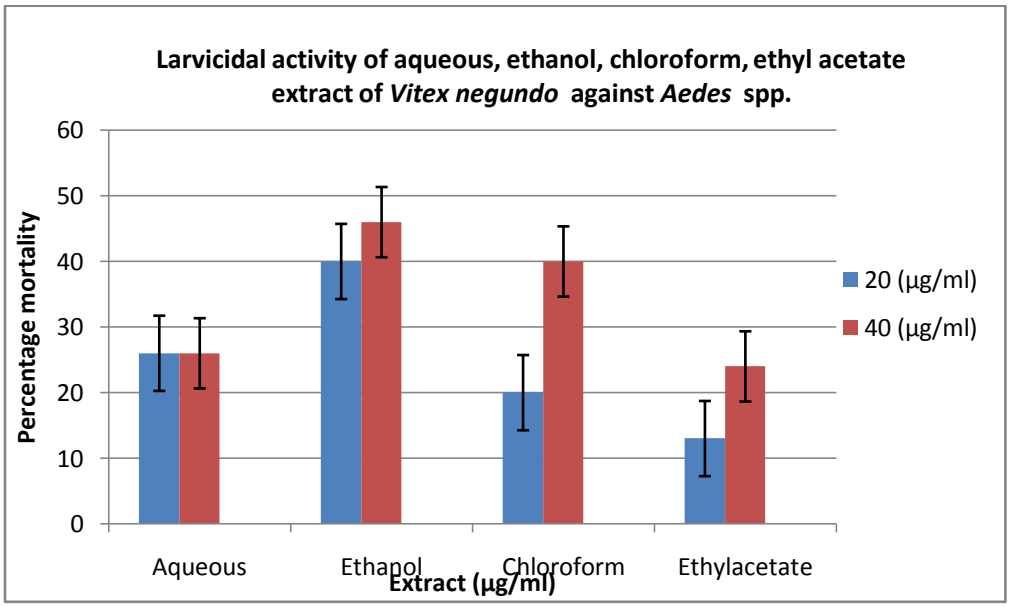
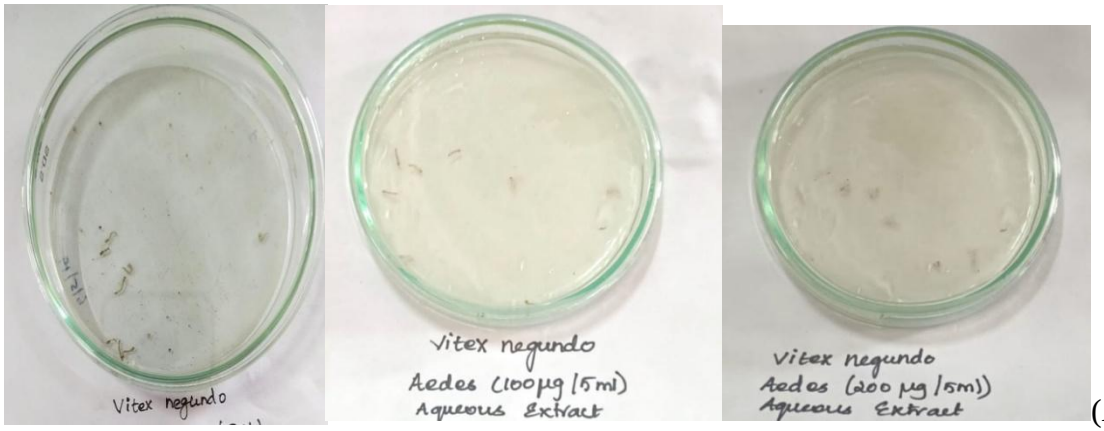
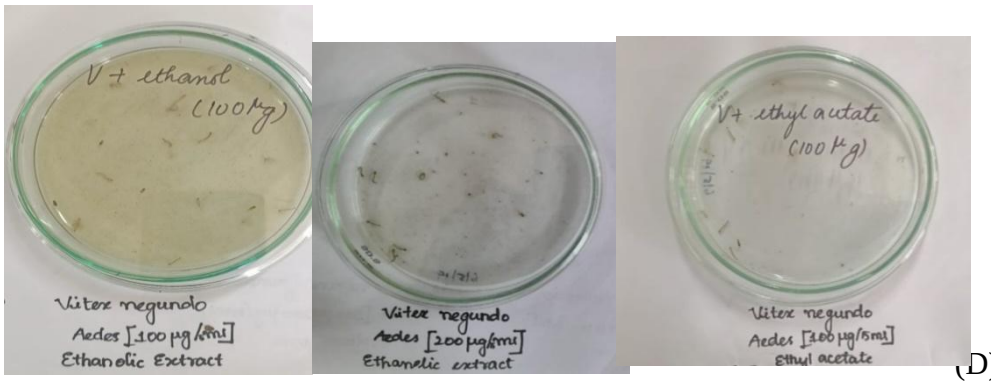


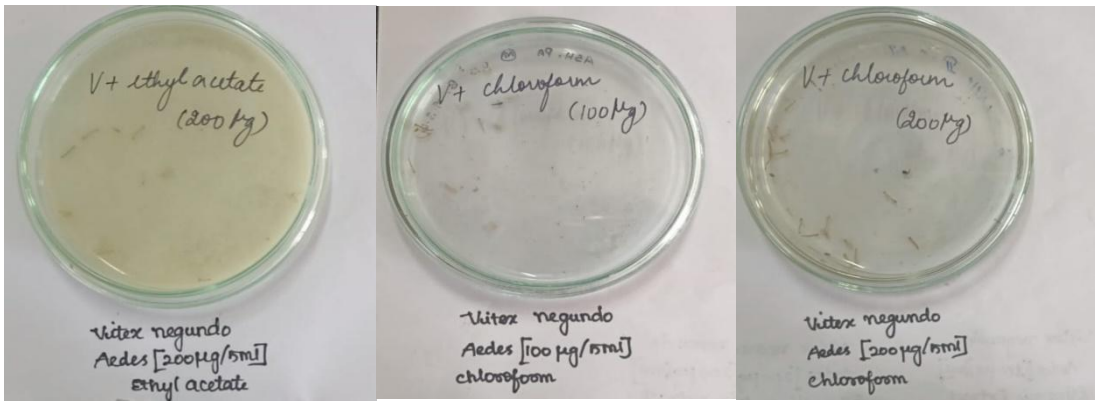
Figure: 13 - Larvicidal activity of aqueous, ethanol, chloroform, ethyl acetate extract of *Vitex negundo leaves*against *Aedes spp.*



(A) - distilled water(B) - Aqueous extract- 20µg/ml(C) - Aqueous extract- 40µg/ml



(D) Ethanolic extract - 20µg/ml(E) Ethanolic extract - 40µg/ml (F) Ethyl acetate20µg/m



(G) Ethyl acetate - 40µg/m (H) Chloroformextract - 20µg/ml (I) Chloroformextract - 40µg/ml

(A) Distilled water (B) - Aqueous extract- 20µg/ml (C) - Aqueous extract- 40µg/ml

(D) Ethanolic extract - 20µg/ml(E) Ethanolic extract - 40µg/ml (F) Ethyl acetate - 20µg/ml

(G) Ethyl acetate - 40µg/ml (H) Chloroformextract - 20µg/ml (I) Chloroformextract - 40µg/ml

Table 4.1.a showed the larvicidal activity test of the leaf extract of *Vitex negundo*in which ethanol extract showed the highest degree of mortality of 66% at (40 µg/ml) and 60% at (20µg/ml) followed chloroform showed the mortality of 40% at (40 µg/ml) and 20% at (20µg/ml) and aqueous extract showed the mortality of 26% at (40 µg/ml) and 26% at (20µg/ml) and the least mortality was shown by ethyl acetate of 24% (40 µg/ml) and 13% at (20 µg/ml).

Table:4.1.b- Larvicidal activity of aqueous, ethanol, chloroform, ethyl acetate extract of *Vitex negundo* leaves against *Culex spp.*

Sample tested	Concentration (µg/ml)	No. of larvae		Time duration (hours)	Percentage mortality
		Exposed	Survived		
Distilled water	-	15	0	24	0
Aqueous extract	20	15	11	24	26
	40	15	12	24	24
Ethanol extract	20	15	10	24	33
	40	15	10	24	33
Chloroform extract	20	15	11	24	26
	40	15	10	24	33
Ethyl acetate extract	20	15	7	24	53
	40	15	9	24	40

Figure: 14 - Larvicidal activity of aqueous, ethanol, chloroform, ethyl acetate extract of *Vitex negundo* leaves against *Culex spp.*

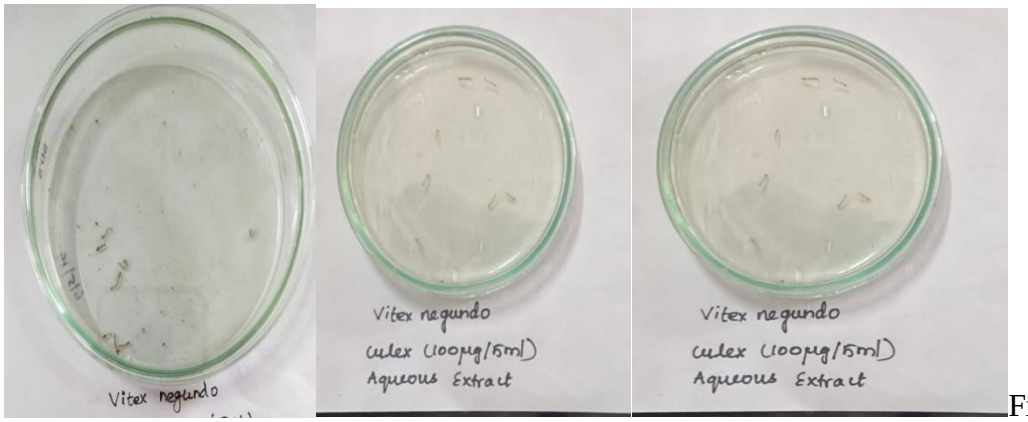
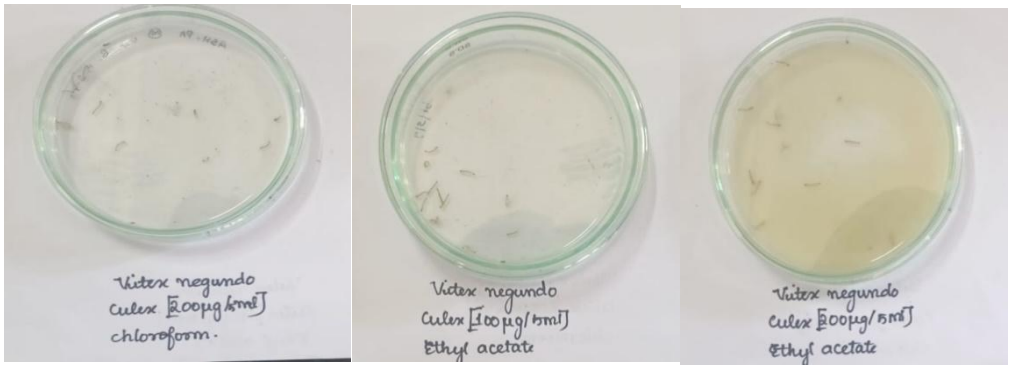


Figure: 14 (A) - distilled water (B)Aqueous extract - 10µg/ml (C) - Aqueous extract - 20µg/ml

(D) - Ethanolic extract -10µg/m(E) -Ethanolic extract -20µg/ ml (F) Chloroform -10µg/ml



(E) Chloroform - 20µg/ ml (G) Ethyl acetate - 10µg/ml (H) Ethyl acetate - 20µg/ml

(A) Distilled water (B) Aqueous extract - 10µg/ml (C) - Aqueous extract - 20µg/ ml

(D) Ethanolic extract -10µg/ml (E)Ethanolic extract -20µg/ ml (F) Chloroform -10µg/ml

(E) Chloroform - 20µg/ ml (G) Ethyl acetate - 10µg/ml (H) Ethyl acetate - 20µg/ml

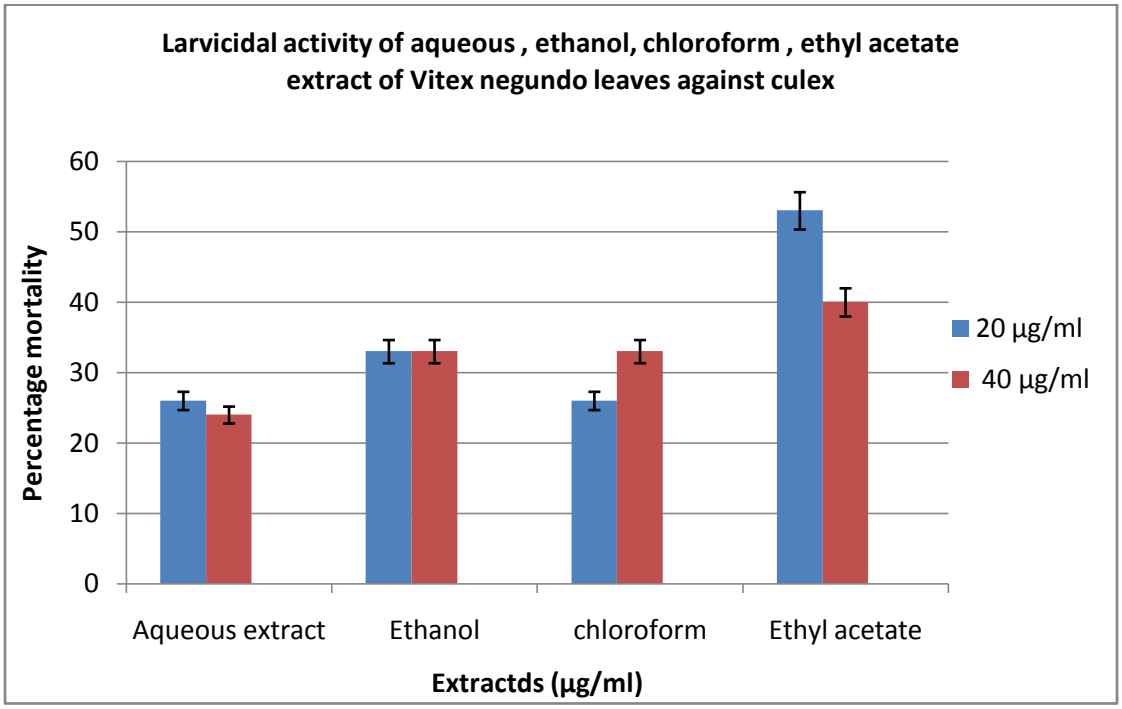


Figure: 15- Larvicidal activity of aqueous, ethanol, chloroform, ethyl acetate extract of *Vitex negundo*against *Culex spp.*

Table 4.1.b showed the larvicidal activity test of the leaf extract of *Vitex negundo*in which ethyl acetate extract showed the highest degree of mortality of 53% at (40 µg/ml) and 40% at (20µg/ml) followed ethanol showed the mortality of 33% at (40 µg/ml) and 33% at (20µg/ml) and chloroform showed the mortality of 33% at (40 µg/ml) and 26% at (20µg/ml) and the least mortality was shown by aqueous extract of 26% (20 µg/ml) and 24% at (40 µg/ml).

Karunamoorthi, 2009 evalutatedlocal rural population are widely using leaves of *Vitex negundo* to repel insects by mixing leaves with grain stores and fresh leaves are burnt with grass as a fumigant against mosquitoes. *Vitex negundo* leaves are available throughout the year, at the same time as seeds are available only in particular months. (Karunamoorthi, 2009)

In another study, Teklani et al., 2017reported thatthe volume of 40.0 mL of distilled water was added into clean 100 mL beakers. Then to each beaker, eight mosquito larvae (*Aedes*

aegypti) were added. A volume of 1 ml of plant extract was added to each beaker and the mortality of larvae was observed within 24 hours. A preliminary larvicidal screen was investigated with all the leaf extracts and triplicates were carried out for the extracts which showed a high mortality percentage

In this present study the larvicidal activity of *Aedes spp.* and *Culex spp.* was checked using the extract prepared using aqueous, methanol, chloroform and ethyl acetate extract at the rate of 20µg/ml and 40µg/ml. The ethanolic extract has shown highest mortality of 66% against *Aedes spp.*larvae in the concentration of 40µg/ml. While the ethyl acetate highest shown highest mortality of 53% against culex larvae in the concentration of 20µg/ml.

4.2 Mosquito repellent activity:

4.2.1 Cage test:

Table: 4.2.1 – Efficacy of repellent in Cage test for *Aedes spp.* mosquitoes

Incense bar Prepared using	**No. of mosquito exposed**	**No. ofmosquito died after**		
		1 hour	**2 hour**	**3 hour**
Herbal base.	**20**	**0**	**2**	**2**
Herbal base and panchakavyam.	**20**	**2**	**3**	**5**
Herbal base, Panchakavyam and *Vitex negundo* leaves	**20**	**5**	**7**	**7**

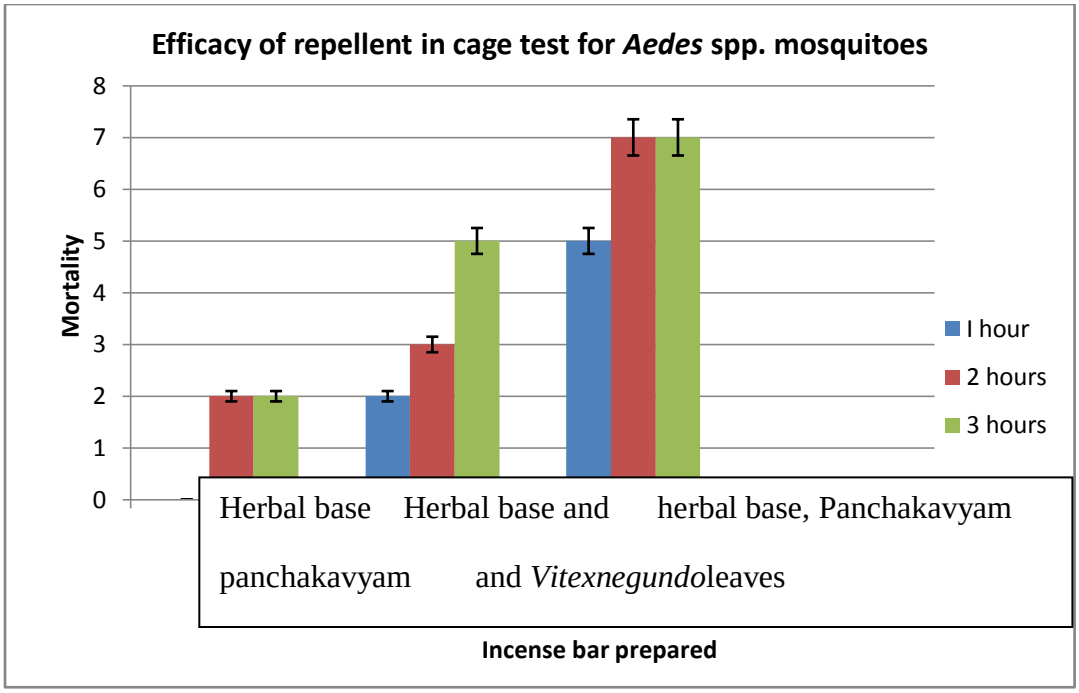


Figure:16 – Graphical representation of Efficacy of repellent in cage test for *Aedes spp.*

(A) Incense bar using herbal base(B) Incense bar using herbal base and panchakavyam

(C) Incense bar using herbal base, panchakavyam and *Vitex negundo* leaves

Figure: 17- Efficacy of repellent in cage test for *Aedes spp.*

Table 4.2.2 Efficacy of repellent in Cage test for *Culex spp.* Mosquito

Incense bar prepared using	**No. of mosquito exposed**	**No. of Mosquito died after**		
		1 hour	2 hours	3 hours
Herbal base.	**20**	**0**	**3**	**3**
Herbal base and panchakavyam.	**20**	**2**	**2**	**4**
Herbal base, panchakavyam and *Vitex negundo* leaves powder.	**20**	**6**	**6**	**7**

(A) - Incense bar using herbal base (B) Incense bar using herbal baseand panchakavyam

(C) Incense bar using herbal base, panchakavyam and *Vitex negundo*leaves

Figure: 18 - Efficacy of repellent in Cage test for *Culex spp.* Mosquito

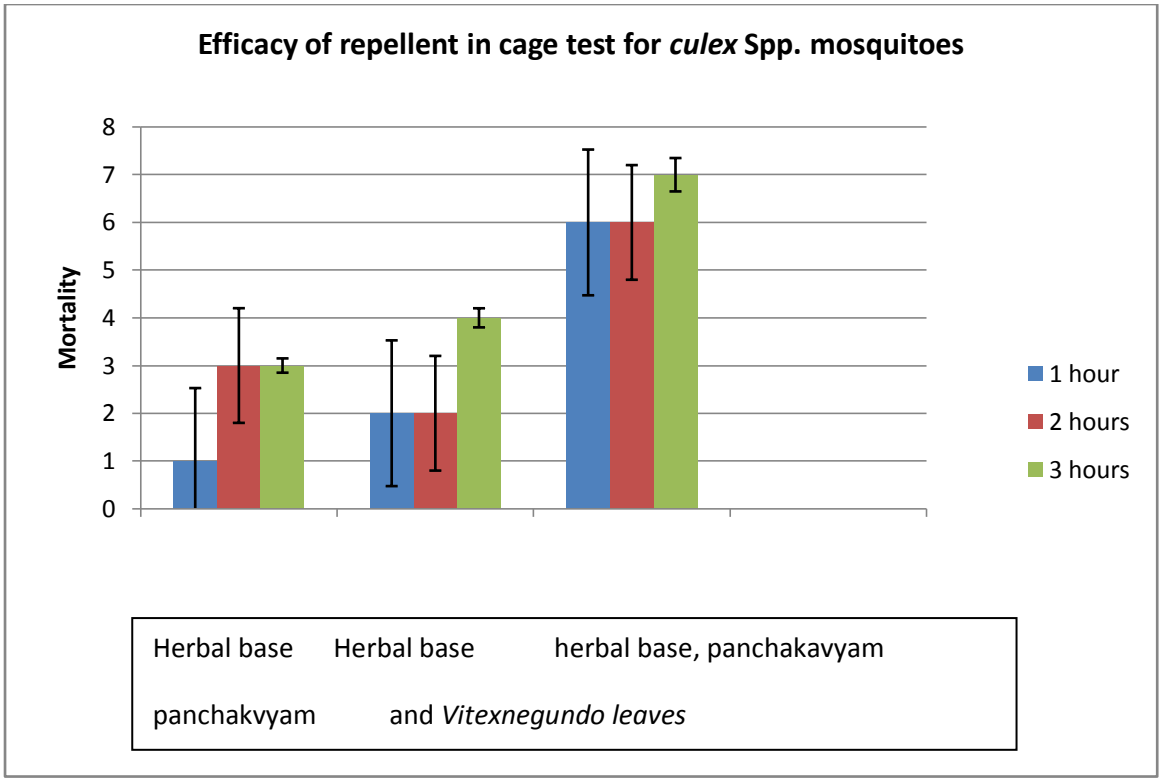


Figure: 19 – Graphical representation of efficacy of repellent in cage test for culex mosquito

Ponkiya *et al*., 2018 reported thatcommercial mosquito repellents contain various synthetic chemicals which have been shown to be toxic to the uses. Of recently plant based products are being explored for human welfare in addition to food.

Watanabe *et al*., 1995researched ona new natural mosquito repellent was isolated from fresh leaves of *Vitex rotundifolia*. Its structure was elucidated by an extensive nuclear magnetic resonance spectral analysis to be a cyclopentene dialdehyde named rotundial. This compound possessed potent repelling activity against *Aedes aegypti*.

Mehlhorn *et al*., 2005reported thatthe repellent efficacy of leaf extract of *Vitex negundo* is also comparable with CO_2 extract of the seeds of the Mediterranean plant *Vitex agnuscastus* (monks pepper).The above-cited studies undoubtedly indicated that Vitex plants possess promising potent repelling activity against mosquitoes and other bloodsucking insect.

Logan*et al*., 2010studied that *Anopheles gambiae*and *Cx. quinquefasciatus* mosquitoes were laboratory-reared at icipe, Kenya. Fresh cages (50 × 50 × 50 cm), with 50 female mosquitoes in each, were used for each treatment within a testing session. Repellent compounds in acetone (solutions (0.5 ml) were applied to a volunteer's forearm from the elbow to the wrist and the hand was covered with a Nitrile glove. Acetone (or ethanol) alone (0.5 ml) served as

a control on the other arm. The control arm was inserted into a cage and the number of landings was recorded over 3 min. Then the treatment arm was inserted into the same cage and the number of landings recorded in the same way. Control and treatment arms were interchanged between experimental sessions to eliminate bias.

The present study indicates that incense bar using herbal products, the incense bar withherbal products and panchakavyam and the incense bar with herbal products, panchakavyam and *Vitex negundo* leaves kill 4, 10, 19 *Aedes spp.*mosquitoes in 3 hours respectively. The incense bar using herbal products,the incense bar with herbal products and panchakavyam and the incense bar with herbal products, panchakavyam and *Vitex negundo* leaves kill 6, 8, 19 culex mosquitoes in 3 hours respectively. With these observations, we can say that *Vitex negundo* plant displays mosquito repellent activity and it can be utilized to produce eco-friendly and cost effective mosquito repellent products in the form of incense bar.

4.2.2EXCITO- CHAMBER TEST:

4.2.3. Table – Efficacy of repellent in excito - chamber test for *Aedes spp.* mosquitoes:

Time (hours)	NEX	NES	NDE	Mosquito repellence %
1	20	2	2	20
2	20	4	2	30
3	20	6	4	50

Figure: 20- Efficacy of repellent in Excito- chamber test for *Aedes spp.*

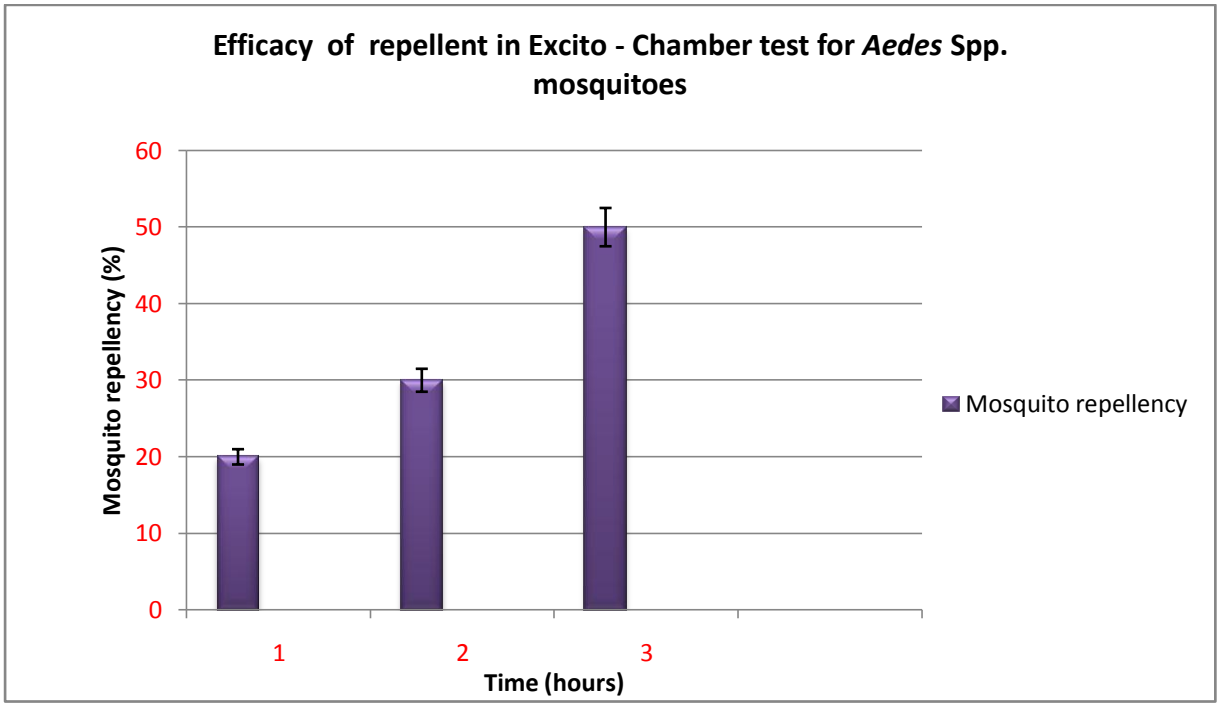


Figure: 21 - Graphical of representation Efficacy of repellent in Excito- chamber test for *Aedes spp.*

4.2.4 Table – Efficacy of repellent in excito - chamber test for *Culex*spp. mosquitoes:

Time (hours)	NEX	NES	NDE	% Mosquito repellence
1	20	3	3	30
2	20	6	2	40
3	20	8	4	45

Figure: 22 - Efficacy of repellent in Excito – chambertest for *Culex Spp.* mosquitoes

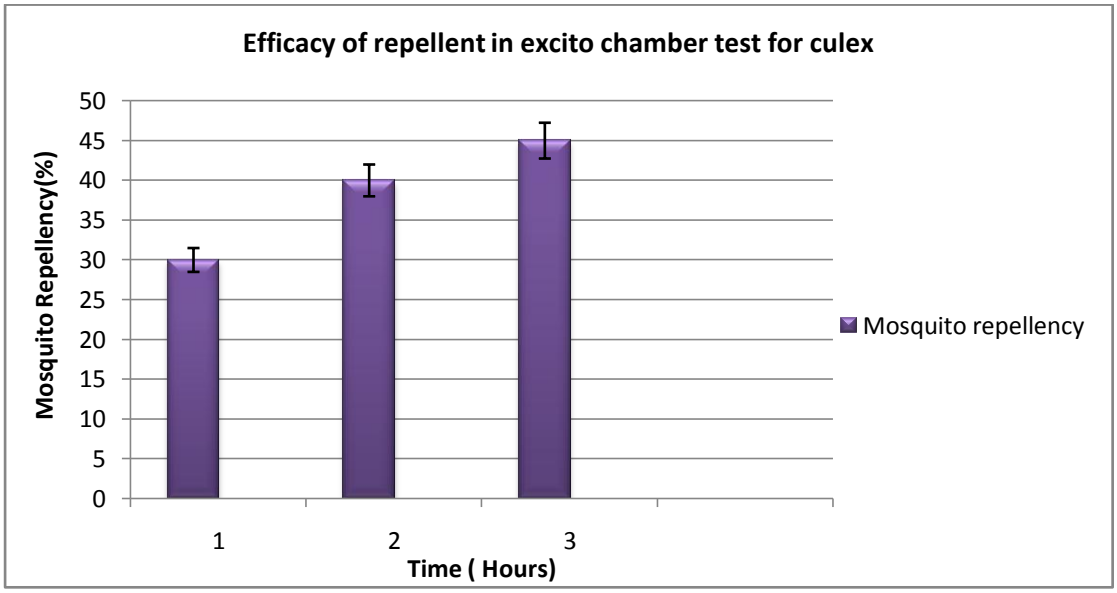


Figure: 22 – Graphical representation of efficacy of repellent in excito – chamber test for *Culex spp.*mosquitoes

Ponkiya *et al*., 2018 studied that commercial mosquito repellents contain various synthetic chemicals which have been shown to be toxic to the uses. Of recently plant based products are being explored for human welfare in addition to food.

Karunamoorthi et al., 2008 reported thatplants 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 and other bloodsucking insects. We are all just around the corner to reinstate the chemical substances with plant-derived ones. In the present investigation, we have identified ecofriendly substances of leaves

of *Vitex negundo* for the control of vector mosquitoes. Plants can provide safer alternatives for modern deadly poisonous synthetic chemicals.

The present study indicates that incense bar usingherbal products, panchakavyam and *Vitex negundo* leaves show 20% repellence in 1 hour, 30% repellence in 2 hours and 50% repellence in 3 hours against *Aedes spp.* The incense bar usingherbal products, panchakavyam and *Vitex negundo* leaves show 30% repellency in 1 hour, 40% repellence in 2 hours and 45% repellence in 3 hours against *Culex spp*.

4.2.3 Comparative studies:

Table 4.2.5 Comparative studies for commercially available mosquito coil and *Vitex negundo* computerincense bar

Mosquito coil	No. of mosquitoes exposed	Time (hours) of exposure	No. of mosquitoes dead
Goodnight	**25**	**1**	**25**
Maxo	**25**	**1**	**25**
***Vitex negundo* Computer incense bar**	**25**	**1**	**20**

(A) – Good night mosquito repellent activity by cage test

(B) – Maxo mosquito repellent activity by cage test

(C) -*Vitex negundo* incense bar mosquito repellent activity cage test

Figure: 23-Comparative studies for commercially available mosquito coil and *Vitex*

***negundo* computerincense bar**

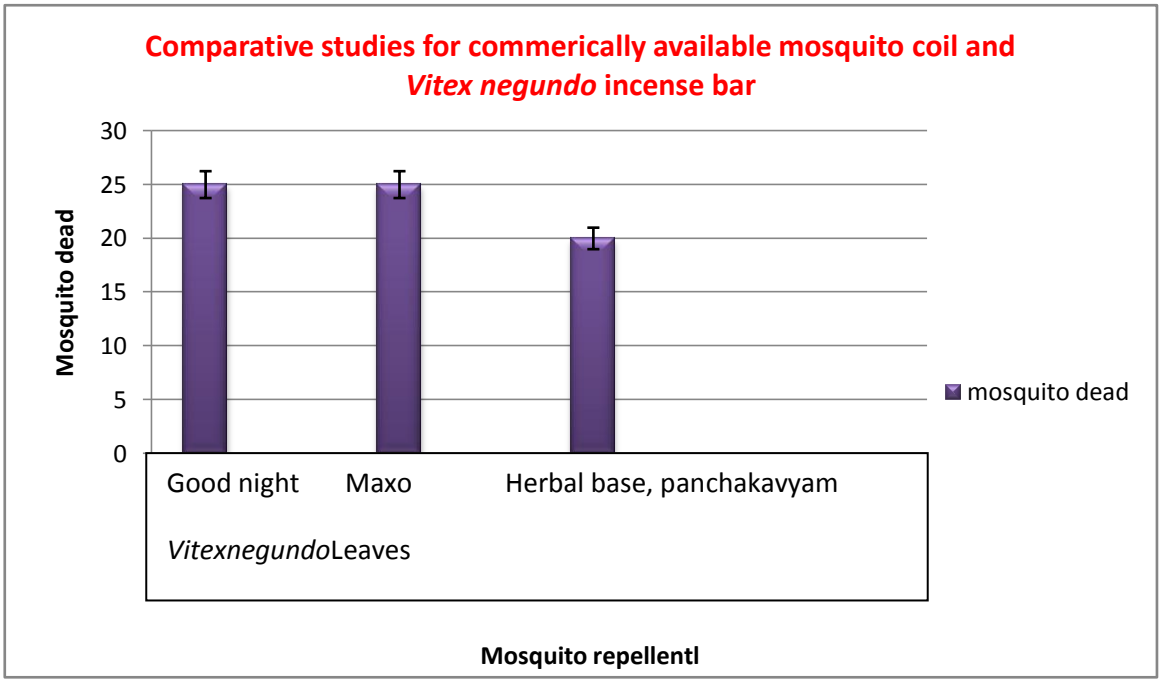


Figure 24- Graphical representation ofcomparative studies for commercially available mosquitocoil and *Vitex Negundo* computer incense bar

Karunamoorthi*et al*., 2016 reported thatherbal pesticides gained importance recently; plant products are considerably safer and eco-friendly for pest control even before the introduction of chemical pesticides; detailed knowledge concerning them is still lacking. As a result, it is the hour to launch extensive search to explore eco-friendly biological materials for control of insect pests.

Madhubabu *et al*., 2012 studied that in rats exposed to allethrin-based mosquito coil smoke for 15–180 days, compared to the unexposed controls, lipid peroxidation was increased in the cauda and testes. Histopathological analyses revealed loss of tubule architecture, epithelial cell disruption, increase in lumen size, interstitial edema, and presence of dead spermatozoa. p53 gene expression was differentially altered in the epididymis and testes. The expression of spermatogenic factors, namely, stem cell factor and its ligand c-Kit was unaltered though decreased levels of Tgf-β1 were observed. Results of this study demonstrate that prolonged exposure to allethrin-based mosquito coil smoke could lead to oxidative stress and compromise germ cell production.

Garba *et al*., 2007 revealed thatthis study was carried out to investigate the effect of inhaling mosquito coil smoke on the histomorphology and biochemistry of the rats' kidney. At the end

of each experimental period, blood was obtained 3 from each rat for the determination of serum levels of Na+, K+, Cl- , HCO, Urea and Creatinine. The rats were then sacrificed and the kidneys obtained were processed for routine histological analysis. Biochemical analysis of blood serum showed a significant increase in the levels of urea and creatinine in rats exposed to the mosquito coil smoke but serum levels of Na+, K+, Cl- and HCO were – not affected significantly. Histopathological assessments of the kidney tissues of the rats exposed to mosquito coil smoke were severe multifocal congestion, cystic dilatation in the medulla; proteinaceous casts within ducts, interstitial mononuclear cellular infiltration and widespread fibrosis(Garba *et al*., 2007).

This study reveals that computer incense bar equally kill the mosquitoes of the commercially available mosquito coil. In the present investigation, we have identified eco-friendly substances leaves of *Vitex negundo* for the control of vector mosquitoes. Plants can provide safer alternatives for modern deadly poisonous synthetic chemicals.

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

The indiscriminate used of synthetic chemicals and insecticides to control mosquitoes in their natural habitat has developed a lot of resistance and inevitable environmental related hazard which bring about negative eco-degradation. Current research of mosquito repellent activities is geared towards environmentally safe and non-hazardous botanicals as insect repellent to target organisms. The current research that uses the chemical constituents and biological activity of *Vitex negundo* were reviewed recently. Although the volatile oil from the leaves has shown mosquito repellent activity, no active principle has been isolated. The leaf extract possess an antiarthritic effect and smoke of its leaves is used to get relief from headaches. In search of new mosquito repellents from Indian plants, we examined the leaves of *Vitex negundo*, which is known to have potential for pest control and insect repellence.

Local rural population are widely using leaves of *Vitex negundo* to repel insects by mixing leaves with grain stores and fresh leaves are burnt with grass as a fumigant against mosquitoes. *Vitex negundo* leaves are available throughout the year, at the same time as seeds are available only in particular months.

With this background the present study was designed, to check the larvicidal activity on *Aedes spp.* and *Culex spp.*larvae using the various extract of *Vitex negundo*. The extract which have been used are

- Aqueous extract
- Ethanol extract
- Chloroform extract
- Ethyl acetate extract

To prepare the mosquito repellent incense bar by using the leaves of *Vitex negundo* and compared their efficacy by performing two tests

- Cage test
- Excito - chamber test

Comparing the herbal incense bar with commercially available synthetic repellents like good night and maxo.

The results obtained are summarized below:

By checking the larvicidal activity it is found that the ethanolic extract shown high mortality rate against *Aedes spp.*of 66% at 40 µg/ml and ethyl acetate shown high mortality rate against culex of 53% at 20 µg/ml when it is compared with the other extracts like aqueous, chloroform and ethyl acetate extract.

Repellency test for *Aedes spp.* mosquito species by cage test reveals that incense bar with herbal base, incense bar with herbal base and panchakavyam and incense bar with herbal base, panchakavyam along with *Vitex negundo*leaves kills 2, 5, 7 mosquitoes in 3 hours respectively. Similarly, for *Culex spp.* mosquito species the incense bar with herbal base, incense bar with herbal base and panchakavyam and incense bar with herbal base, panchakavyam along with *Vitex negundo* kills 3, 4, 5 mosquitoes in 3 hours respectively. From this observation we can say that *Vitex negundo* possess strong mosquito repellent activity and it can be utilized to produce ecofriendly and cost effective mosquito repellent products in the form of incense bar.

The excito - chamber method is to observe the mosquito behaviour change in the form of moving away from area with incense bar to the area without incense bar. The present study indicates that incense bar usingherbal products, panchakavyam and *Vitex negundo* leaves show 20% repellency in 1 hour, 30% repellency in 2 hours and 50% repellency in 3 hours against *Aedes spp.* The incense bar usingherbal products, panchakavyam and *Vitex negundo* leaves show 30% repellency in 1 hour, 40% repellency in 2 hours and 45% repellency in 3 hours against culex.

By comparing the herbal incense bar and synthetic repellents it was found that after 1 hour of exposure, the mortality rate is higher for the synthetic repellents than the herbal incense bar. However, it is safe and cost effective.

Conclusion

To conclude, it has been observed that the herbal incense which was made using *Vitex negundo*leaves has a efficiency as a mosquito repellent and the mortality rate of the mosquitoes are also significant. In order to get the 100% mortality rate in future the compounds of plant extract can be studied further in detail and by using the technology the efficiency of the extract can increased so that it can be used in the mosquito repellent cream, lotion etc.

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