Study on mosquito repellent activities of *Eucalyptus globulus* and its herbal computer incense bar

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(20PBC015)

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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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Signature of the Head of the

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1.INTRODUCTION

1.MOSQUITOES

Mosquito- family culicidae, any of over 3,500 species of familiar insect in the fly order Diptera that are essential in public health due to the females bloodsucking activities.yellow fever, zika fever, dengue fever are all diseases spread by mosquitoes (Dahmana *et al.*,2020).

1.1PHYSICAL FEATURES

The veins of the wings and the slender, elongated body of adult are covered in scales. Mosquitoes have extended, piercing mouthparts and have lengthy, fragile-looking legs. Males have bushier feathery antennae than females. Nectar and other plant juices are consumed by the males and females. Females in most animals on the other hand, require proteins from a blood meal to mature their eggs. Mosquito species have preferences and, in certain situations, strong host animals constraints (Ndenga *et al* 2017).

The eggs are placed on the water's surface and hatch into aquatic larvae, also known as wrigglers, which swim in a jerking, wiggling motion. The larvae of most mosquito species feed on algae and algae and organic waste, although a few are predatory and may feed on other mosquitoes. Tumblers, the larval stage of mosquitoes, are energetic and free-swimming unlike most mate. Depending on the species, the length of the life cycle varies substantially (Unlu *et al.,* 2011).

Lactic acid, carbon dioxide, body heat and movement are all thought to attract mosquitoes to their hosts. The high frequency of the mosquito's wing beats causes the hum, and the female's wing beats frequency may be used to determine sex (Amerasinghe *et al., 1995).*

Researchers have looked into modifying mosquito populations to prevent viable mosquito progeny from being produced, hence diminishing mosquito populations. Researchers have also discovered a method for genetically engineering male mosquitoes to pass a gene to their offspring that causes them to die before sexual maturity. Female mosquitoes are less attracted to people were exposed to tiny molecules related to the

neurotransmitter molecule neuropeptide Y, according to researchers. These compounds could be emitted by dispensers in mosquito-infested areas to help prevent mosquito bites(Schreiber,2022).

1.3.MOSQUITO BORNE DISEASES

The development of mosquito borne diseases such as dengue fever, yellow fever, and chikungunya around the world has reignited interest in and a need for active monitoring programmes of common and neglected insect-borne human infectious diseases. Early discovery of infected arthropods can help stop epidemics before they spread and have serious public health repercussions. Mosquito-borne diseases account for around 17% of the total burden of all infectious diseases, according to the World Health Organisization (WHO). Mosquitoes are blood-sucking dipterans in the Culicidae family, and there are now 3583 genuine species documented world wide (Hemingway et *al*., 2006).

Mosquitoes spread a number of medical and veterinary diseases, including malaria, filariasis, encephalitis, yellow fever, dengue fever, rift valley fever, rift valley and others. Malaria is most well known mosquito-borne disease in the world, with about half of the world's population living in malaria-prone areas. zika virus and chikungunya are the remaining arboviral illness that cause severe symptoms or high fatality in humans. Apart from these well known vector-borne disease, mosquitoes are also involved in the transmission of a wide range of pathogens that affect both humans and animals, including west Nile virus (flaviviridae,flavivirus), Rift valley fever (Phenuiviridae, Phlebovirus),Wesselbron virus (flaviviridae, flavivirus), Middelburg virus (Togaviridae, Alphavirus),Wuchereria bancrofti many of these diseases are less common in people and largely affect animals,and there is currently a lack of information on their occurrence and distribution in various Afrotropical habitats (Bamou et *al.*, 2021).

The mosquito vectors of the dengue and malaria pathogens are very climate-sensitive. Metrological circumstances have a direct impact on vector reproduction and mortality rates, and thus population distribution and abundance. Furthermore, they have an indirect impact on epidemiologically crucial characteristics such as the vector's blood feeding frequency and the and pathogen's extrinsic incubation period. In mosquito-borne diseases, these effects frequently result in predictable annual cycling or seasonality. In addition to seasonal

variations, there are also huge surges in clinical load, or “epidemics”, which frequently overwhelm health-care resources. Periodic superannuation in incidence has been reported in endemic disease settings in a number of vector-borne illnesses (Schaffner *et al.*,2021).

1.4.MOSQUITO REPELLENT

Mosquito bites can be uncomfortable, and they can spread vector diseases like dengue fever or malaria to humans. Mosquitoes are drawn to human blood because it contains protein, allowing them to grow their eggs and spread the vector illness from one human to another. the mosquito genus aedes is responsible for transmitting the vector of dengue fever all across the world, with billions of people infected and mortality cases reported. There are only a few vaccinations available to treat viruses spread by mosquito bites; however, researchers have yet to discover a vaccine for dengue fever. The simplest approach to avoid mosquito bites is to avoid them. Repellents are useful products that can be used in a variety of situations. Repellents are practical and cost-effective tools for preventing mosquito-borne illness transmission (Andersen *et al.,* 2017).

Repellents can be natural sources (plants) and chemical sources, DEET (N,N-diethyl-m-tolumide), along with other synthetic repellents like Permithrin, Alletherin,and Malathion, has been demonstrated to be the most effective commercial repellent composition used in the lotions, gels, solution, cream and aerosols.(Adeela*et al.,2016*)

Synthetic repellents such as DEET and other synthetic repellents, on the other hand, have been shown to have a harmful impact on humans. Permithrin,for example, is hazardous in high amounts and can cause dermatitis if it is constantly applied to the skin.(Adeela *et al.*,2016)

Despite the fact that synthetic pyrethroids are used as active ingredients in mosquito repellents all over the world due to their low toxicity in animals, large dosages cause neurotoxic symptoms such as tremors, loss of coordination, hyperactivity, paralysis, and increase in body temperature. Others adverse effects include irriration of the skin and eyes, reproductive consequences, mutagenicity, immune system changes, and so forth(Fathy *et al.*,2017)

Pyrethroid insecticides have been shown in recent years to diminish sperm count and motility, cause sperm head deformity, increase the number of defective sperm, damage sperm DNA, induce anueploidy, influence sex hormones levels, and cause reproductive toxicity.

Pyrethroid exposure levels and potential risks during the usage of other types of regularly used mosquitio repellents are unknown (Rodriguez *et al.*,2015).

Plant –based repellents have been utilized as a personal protection measure against host-seeking mosquitoes for generations in traditional practice. Consumers have recently become more interested in commercial repellents containing plant based components, which are often seen as "safe'' in comparison to long established synthetic repellents, however this is not always the case. To date insufficient investigations have followed the standard WHO pesticide evaluation scheme guidelines for repellent testing. More standardised research are needed to better analyse repellent chemicals and develop new products that offer great repellency.(Ferreira *et al.,* 2011)

Man has been using the repellency of plant material for thousands of years, most simply by hanging bruised plants in buildings, a practice that is still widely used in developing nations. Plants have also been utilised for ages as crude fumigants, in which plants were burned to keep mosquitoes at bay. Plant-based repellents are still widely used in this traditional way throughout rural communities in the tropics because they are the only means of protection from mosquito bites available for many of the poorest communities. Plants are seen as a safe and reliable way to prevent mosquito bites, therefore "natural" scented favoured (Ferreira *et al.,* 2011).

Plant derived insecticides are an alternative to synthetic pesticides since they produce less pollution and are less hazardous and other benefits *Eucalyptus globulus* is a tree that generally grows to a height of 45 meters, but can either be a stunted shrub or grow as tall as 90-100 meters under ideal condition.*Eucalyptus globulus* is one of the notable medicinal plant that found have insecticidal capabilities. The goal of the study was to look into the repellent qualities of *Eucalyptus globulus*leaf extract against *Aedes* Spp. and *Culex* Spp. mosquito species (Bhuwan *et al.,* 2012).

In search of new mosquito repellents from Indian plants, we examined the leaves of *Eucalyptus globulus* which is 2011) known to have potential for pest control and insect repellency. In the present study we report making of computer incense bar from *Eucalyptus globules* and other 34 herbal products for the first time.

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

- To Prepare of the aqueous extract, ethanolic extract,chloroform extract and ethyl acetate extract with *Eucalyptus globulus* and checking their larvicidal activity on the species of *Aedes* Spp. and *Culex* Spp.
- To prepare computer incense bar with herbal products, herbal products with panchakavyam, herbal products, panchankavyam along with *Eucalyptus globulus* leaves.
- To check the mosquito repellency of the computer incense bar prepared usingherbal products, herbal products with panchakavyam, herbal products, panchankavyam along with *Eucalyptus globulus* leaves against the mosquito species of *Aedes* Spp. and *Culex* Spp.
  1. Cage test
  2. Excite-chamber
- To compare the *Eucalyptus globulus* 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 the literature is presented in the following chapter.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Mosquito borne disease

Mosquitoes are for transmission of more diseases than any other arthropod group. Mosquito-borne infections continue to be a serious public health issue in both human and veterinary medicine (Bagavan *et al.,* 2011).

Many of the vector-borne disease that harm humans and other animals are transmitted by mosquitoes. Mosquitoes, as vectors of dangerous human disease, are major public health(Hag *et al.,* 2004).

The West Nile virus is transmitted by Pipiens mosquito. Encephalitis or meningitis is a condition that affects the brain and spinal cord. Eventually, brain tissue is destroyed,resulting in lasting neurological damage. There are several mosquito species that belong to the genera *Anopheles* Spp. Culex,and *Aedes Spp* are vectors for diseases such as malaria, filariasis, and dengue fever. The main vector of Aedes aegypti is dengue fever is also known as dengue hemorrhagic fever (Mohan *et al.*,2007).

Lymphatic filariasis is a parasitic infection that affects at least 120 million people in 173 countries across Africa, India, Southeast Asia, and the Middle East. Islands in the Pacificocean. These illness not only create a lot of pain, but they also cause a lot of stress. Not only do they increase morbidity and death, they also cost lot of money and social upheaval in developing countries like India, china, for example. India alone accounts for around 40% of global GDP.The burden of filariasis and the anticipated annual economic loss is around 720 crores (Bagavan *et al*., 2017).

2.1.1.MOSQUITO SPECIES CAUSES MOSQUITO-BORNE DISEASES

AEDES

Hundreds of arthropod-borne viruses (arboviruses) have been identified, with roughly 30 causing disease in humans (Cleoton *et al.,* 2012). Despite this diversity, only four arboviruses, yellow fever, dengue fever, chikungunya, and zika have caused significantly more human suffering. *Aedes aegypti*, a mosquito, has been the dominant vector in practically all significant human epidemics of these four viruses in the past. Because these

viruses are endemic to Africa, humans are a native African primate, and *Aedes aegypti* is a native African mosquito. It has been postulated that the viruses, mosquitoes, and primate hosts evolved together over time in their native Africa before spreading around the world.

These four viruses are single RNA viruses with high mutation rates, which has facilitated their rapid development and adaption to replicate in many hosts. Dengue viruses (DENVs), zika viruses (ZIKV), are flaviviruses, chikungunya virus is an alplavirus (CHIKV) in humans, they all induce a high temperature that lasts 4-14 days and point pain. However, each has its own pathology, with yellow fever virus (YFV) and DENVs having significant fatality rates, but CHIKV and ZIKV have very low rates(Jayme *et al.*, 2019).

CULEX

Culex mosquitoes are the most common mosquito species on the planet. They are known to feed on humans and animals, abehaviour that boosts their ability to transmit zoonotic infections and makes them a significant public health danger. *Culex* Spp.have adapted to human-made habitats over decades. The*Culex pipiens* complex, which includes six species: *Culex quinquefasciatus say, Cluex pallens coquillet, Culex australicus Dobroworsky and Drummond, Culex globocoxitus, Dorotworsky, Culex pipiens Lineneaus, and Culex molestus* is one of the important groups in the Culex genus. *Culex pipiens species, particularly Culex quinquefasciatus*, are common and dominating in urban areas, particularly in Africa, where ideal environmental circumstances caused by fast unplanned urbanisation are contributing (Elysee *et al* .,2019).

Culex species can cause nuisance in addition to transmitting diseases such Rift valley fever, West Nile virus, and lymphatic filariasis. The latter, which is caused by the parasite Wuchereria bancrofti, is common in Asia and Sub Saharan Africa and is considered one of the main causes of long-term impairment (Elysee *et al.*, 2019).

Figure 1: *Aedes* Spp.

Figure 2: *Culex* Spp.

PICTURE REFERENCE:

1.https://upload.wikimedia.org/wikipedia/commons/thumb/3/3e/Aedes_aegypti_feeding.jpg/220px-Aedes_aegypti_feeding.jpg

2.https://upload.wikimedia.org/wikipedia/commons/thumb/0/0b/CPipFm.jpg/220px-CPipFm.jpg

2.1.2.CLIMATE CHANGE AND MOSQUITO BORNE DISEASE:

Climate change is clearly having a negative impact on human health, with both direct and indirect consequences, as well as environmental (e.g. safe drinking water) and social determinants (e.g. poverty). These are affecting a big number of people all across the world right now. According to the world Health Organization, there will be an additional 250,000 fatalities worldwide. Between 2030 and 2050, 38,000 extra deaths are expected as a result of climate change.48,000morefatalities due to diarrhoea in older owing to heat exposure. Malaria killed 95,000 people, while childhood malnutrition killed another 95,000 (Reiter *et al.*, 2001).

Tropical and subtropical climate conditions, which are typified by high temperatures, prolonged rainfall, and high air humidity, provide an ideal setting for mosquito survival, development, reduction and replication. For example, irregular rainfall patterns can have a good or detrimental impact on the environment. Outbreaks caused by mosquitoes are becoming more common. Rainfall and flooding occurrence may exacerbate the problem.

Mosquito larvae habitats and possible vector breeding sites, excessive rains, on the other hand causes flash floods, destroying mosquito eggs or washing larvae away. As a viral carrier, Mosquitoes require water breeding places, and a lack of rainfall or extended droughts may result in the larvae desiccation. Rainwater is collected and stored, creating a stagnant water environment for mosquito breeding *(Walter et al.,2019).*

2.1.3.DENGUE

Dengue fever is the one of the most common mosquito-borne in the world .They can be asymptomatic or cause a fever with no discernible cause. Dengue fever, dengue haemorrhagic fever (DHF). Dengue shock synmdrome is kind of dengue fever. Every year, 100 million cases of dengue fever and half million cases of dengue hemorrhagic fever. Children accounts for 90% of DHF participants are less than 15 years. There is no vaccine available for preventing this disease. Early detection and immediate action is must to keep the morbidity and mortality rate minimum (Halstead *et al.,* 2007).

2.1.3.1MOSQUITO VECTORS IN DENGUE INFECTION

Aedes mosquito (*Aedes aegypti, Aedes albopictus, and Aedes polynesiensis*) play a significant role in the spread of dengue fever. Aedes aegypti is the principle and most important vector, but depending on the geographic region, *Aalbopictus* Spp. and A polynesinesis may also be vectors. *A albopictus*, for example, has been discovered to spread dengue fever in Thailand, Sumai Island, India, Singapore, and Mexico. *Aedes aegypti*, a but biting mosquito that breeds in containers, is found in tropical and subtropical climates. They spend much of their time indoors, mostly in living rooms and bedrooms. This increase man-vector contact while reducing contact with insecticides sprayed through doors, making it, more difficult to manage this vector (Halstead *et al.,* 2007).

Polluted water small collections of water, such as flowers vases or coconut shells, are ideal breeding grounds for *Aedes aegypti*. Eggs can live for long time because they can resist extreme temperatures. Garbage disposal that is improper or insufficient, both of these outcomes are made easier by wastewater drainage. Unplanned urbanisation may be to blame for high levels of pollution (Guazman *et al., 2015*).

2.1.4. MALARIA

Malaria is a disease that has affiliated humans since the dawn of humanity. It is still one of the most dangerous infectious disease, with significant rate of morbidity and fatality. The Plasmodium parasite can cause malaria in variety of ways. Most common are *Plasmodium vivax,* which account for more than 95% of all cases identified worldwide, but others include *Plasmodium malariae, Plasmodium knowlesi*. A female Anopheles mosquito transmits malariato humans by inoculating sporozoites into human host. When sporozoites reach human liver cells, they convert into merozoites, which are different from sporozoites. The merozoite enters the erythrocytes through bloodstream and multiplies to make new merozoites. After erythrocytes are broken, some of the merozoites are converted into gametocytes (Antonio *et al.,* 2019).

When an infected person is bitten by an Anopheles mosquito, the merozoites re-enter the body, sexual reproduction occurs inside the mosquito's midgut,where zygotes are produced and oocytes mature. As the expand and break, sporozites are released, which infect the mosquito's salivary glands. After that , parasite will be in the correct shape to a new person (Antonio *et al.*,2019).

2.1.5.CHIKUNGUNYA

Chikunguya fever, an acute illness marked by fast onset of fever, incapacitating polyarthralgia and arthritis, rash, myalgia, and headache, affects the great majority of infected people. Acute CHIKV sickness has symptoms that are similar to dengue fever, and case reports imply that CHIKV epidemics occurred as early as 1779, but were mistakenly assigned to dengue virus. Unlike dengue fever.CHIKV sickness is characterised by recurrent musculoskeletal disease, particularly affecting the peripheral joints, which can last for month to years following an initial infection. CHIKV sickness is frequently self-limiting and has a low fatality rate. But CHIKV infection symptoms that lead to acute and chronic impairment have serious consequences, including a significant impact on infected patient's quality of life as well as significant economic and communal ramifications (Laurie *et al.,*2017).

2.1.6.ZIKA VIRUS

The mosquito- borne zika (ZIKA) belongs to the flavivirus genus, which is part of the flaviviridae family. ZIKV is spreads by various mosquito species, including Aedea africanus, *Aedes aegypti, Aedes aegypti*, *Aedes albopictus* and *Aedes hensili*, like other flaviviruses. In

almost 80% of cases, ZIKV infection goes undiagnosed or is asymptomatic, and the majority of patients have relatively minor symptoms. ZIKV is the most typically spread by mosquito bites, when the virus infects human skin cells directly, infecting permissive human dermal fibroblasts, epidermal keratinocytes, and immature dendritric cells. It is currently regarded as one of the most dangerous illness affecting public health (Kirill *et al*.,2019).

In the light of current situation and the failures associated with mosquitoes invading new territories and causing devastating outbreaks, new tools, molecules, plans, synergistic associations, and mosquito control methods are being developed to help achieve strategic objectives such as protecting at-risk population, particularly in endemic areas, preventing the international spread of mosquitoes and the diseases they carry, and quickly containing epidemics. Some tactics are in the preliminary testing or validation stages, while others have only recently been implemented. Because of their critical efficiency and economical importance, the most pressing need is to create new pesticides to combat mosquito-borne diseases (Handi*et al.,* 2020).

2.2. MOSQUITO HOST INTERACTION

One of the most persistent problems in repellent research is determining how foreign compounds influence insect behaviour. Research necessitate paying attention to the entire range of behaviours and events related with host seeking and feeding over time and geography. As a result, mosquito-host interactions serve as a bridge between suborganismal and organismal investigation of chemical repellents (Agboli *et al* 2019).

Haematophagy carries significant dangers because identification by the host can be lethal to the arthropod in many circumstances. A successful mosquito can find and approach a possible host, assess the quality of the food source, and begin feeding without the host being aware of its existence. This process, which is controlled on multiple levels, is required for mosquito levels, is required for mosquito growth and reproduction. Female mosquito life cycle traits are crucial to explore since they often relate to host-seeking capacity. The difference autogeny and anautogeny is that some female mosquitoes emerge from the pupal stage withenough protein for egg formation, whereas others require a blood meal to meat the nutritional requirements for egg maturation. There is a time interval required for maturation and enhanced sensitivity of the receptors essential for host location in the yellow fever mosquito, *Aedes aegypti* example of an anautogenous female (Valderrma *et al.*, 2017).

Endogenous circadian cycles and hunger state, which are depending on the length of food restriction, affect a mosquito's appetitive seeking state. Its thought be an undirected or even random search that ends when a host stimulus is found. Activation is the name given to this single event. There is enough data to suggest that one enough data to suggest that one specific host cue, carbon dioxide, has a role in both the activation and orientation responses. Other host cues, in addition to carbon dioxide, may play a key role in the action response. Interspecific variation, particularly between anthropophilic and zoophilic mosquito species, is most likely to blame for the differences (King *et al.*, 2012).

Individual mosquitoes will lift off and initiate a persistent flight after being exposed to carbon dioxide. This flight behaviour is known as optomotor anemotaxis. Mosquito upwind flying patterns are similar to other insects the follow a pulsed chemical gradient towards a source, known as orientation. Mosquitoes are assumed to be attracted to their potential host by sight, heat, and smell cues once they are close enough (Gretchen *et al.*,2010).

2.3.MOSQUTO REPELLENT

An insect repellent is a chemical or organic compound that makes the environment within 4cm of human skin so unpleasant for insects, they avoid touch and bite. An insecticide, on the other hand, is a chemical or organic compound, frequently derived from plants, that kills insects by releasing a neurotoxic. Some insect repellents, such as permethrin generated from plants and other synthetic pyrethroids, are also insecticides (James *et al.*, 2016).

The three most important reasons to use insect repellent :1) developing and imported arthropod-borne infectious illnesses pose new hazards to human health; 2) the prevalence of new, capable insect vectors of infectious diseases; and 3) the failure to primarily prevent the transmission of most arthropod-borne infection diseases(James *et al.*, 2016).

2.3.1.SYNTHETIC MOSQUTO REPELLENT

Before world war II, mosquito repellents were, mostly plant based, with citronella oil being the most extensively used compound and the benchmark against which others were evaluated. The synthetic chemical repellents begin to appear around this time. The majority of the commercial mosquito repellents are made with non-biodegradable chemicals like N,N,-

diethyl-3-methylbenzamide (DEET), dimethylphthalate (DMP), and allerthinm, which can pollute the environment and cause severe health hazards if it is used in excessive doses in human (Fathy *et al.,* 2017).

1.DEET

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 has long-lasting impact. The US Department of agriculture discovered DEET as a mosquito repellent, and the US Army patented it in 1946. It was approved for general use in 1957, and since it has become a staple insect and arthropod repellent. DEET is the most researched insect and is commonly used as a positive control when comparing the efficiency of various repellents. The protection provided by DEET is dose dependent: the higher the dosage, the longer the protection. The standard concentration of DEET in commercial products is 20-25%. The mixture played a role in the reduced protection time. Because of its high cost disagreeable odour, and discomfort of continual application on exposed skin at high doses, DEET plays limited role in disease management in endemic areas (Dahmana*et al.,* 2017).

2. PERMETHRIN

Permethrin, a pyrethroid insecticide derived from the Chrysanthemum cinereriifolium plant, is pyrethroid pesticide. 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 clothing, bed, nets, and other items due to its sole position as a contact insecticide via neural toxicity and as an insect repellent. Permethrin-treated clothing is an important arthropod protection against a wide range of bloodsucking arthropods while posing few safety risks, especially whenused in conjugation with other protection stratergies such as applying topical repellents(Thireou *et al.,* 2018).

3.PICARIDIN

Picaridin (1-piperidinecarboxylic acid 2-(2-hydroxyethyl)-1-methylpropylester) is a colourless, odourless piperidine analogue created by Bayer using molecular in the 1980s. KBR 3023, icaridin, hydroxyethyl isobutyl piperidine carboxylate, and sec-butyl-2-hydroxyethyl)-piperidine -1-carboxylate are some of the other names of picaridin. Picaridin has same efficacy as DEET, and a 20 % picaridin spray was found to provide superior protection against three mosquito vectors, *Aedes Spp, Anopheles* Spp, and *Culex Spp,* for

roughly 5 hours than DEET. As a result, application must be repeated every 4-6 hours. When administered at the same dose, investigations found no significant difference between DEET and picaridin, with picaridin having higher persistence. To sustain effectiveness greater concentration of picaridinused in this field (>20%) (Gaddaguti *et al.,* 2017).

4.DEPA

DEPA (N,N-diethyl -2-phenyl-acetamide) is a repellent that was produced about the same notoriety as DEET. DEPA has been shown to repel mosquito vectors such as *Aedes agypti, Aedes albopictus, Anopheles stephensi, and Culex quinquefasciatus*. It has recently regained popularity and potentially become significant competitor to DEET, particularly in underdeveloped countries, due to its low cost (Naseem *et al.,* 2017).

5. ETHYL ANTHRANILATE

Ethyl anthranilate (EA) is a relatively novel entomological compound that has received a lot of attention in repellent research in recent years and is being evaluated as a better alternative to DEET. EA is a new mosquito repellent that works against *Aedes aegypti, Anophles stephensi,* and *Culex quinquefasciatus.* Its spatial repellency was found to be exceptionally efficient against all three mosquito species tested. Ethyl anthranilate produced results thst were comparable to the conventional repellent DEPA. As a result, ethyl anthranilate repellent activity holds promise for producing an effective, safe, and environmentally friendly alternative to currently available toxic repellents for personal protection against various mosquito species (Curtis *et al.,* 2017).

2.4.COMPARISON OF SYNTHETIC REPELLENT

The following is a summary of the comparative efficacy of synthetic repellents: Aedes species bit aggressively and *Aedes agypti,* in particular, proven to be resistant to a variety of repellents. *Aedes albopictus* was less difficult to resist than *Aedes aegypti*. DEET is the most researched insect repellent; at greater concentrations, it was found to be more effective against Aedes species, providing up to 10 hours of protection. Although IR3535 and picaridin were effective against this mosquito genus, their efficiency was on average lower than that of DEET. Anopheles Spp and Culex Spp mosquitoes have had fewer studies done on them. The four main repellents of interest had comparable repellency profiles against

Anophles Spp: DEET gave 5-11 hours on average, IR 3535 4-10 hours, picaridin 6-8 hours of protection. Culex Spp mosquitoes are easy to repel, and each repellent provided enough protection. DEET provided protection for 5-14 hours, depending on product concentration, however the test confirming the efficacy of picaridin and commercial products was stopped after 8 hours of protection (curtis *et al.,* 2017).

2.5.EFFECTS OF SYNTRHETIC REPELLENTS

Since 1946, DEET has been used with few known side effects, most of which were caused by excessive or incorrect repellent use. Its toxicology has been investigated more thoroughly than any other repellent, and it has been found safe for human use, including children, pregnant women, and nursing mothers. Despite the fact that insect repellents containing DEET are safe, some side effects have been reported primarily as a result of improper use, including dermatitis, allergic reaction, neurologic and cardiovascular side effects, and encephalopathy in children. Furthermore, there have been a few reports of systemic toxicity in adults after dermal treatment. The second and third trimester of pregnancy has been known, thanks to very low placental cord concentration following maternal DEET exposure, although animal models show teratogenic consequences. DEET also blocks sodium and potassium ion channels in mammals, which contributes to the numbness of the lips after application. Approval for usage in young children is a contentious subject among countries, with some lower concentration and others preferring higher intensities (Fathy *et al.,* 2017).

Toxicity concerns are negligible when permethrin is impregnated properly in textiles and nets. Despite the fact that synthetic pyrethroids are used as active ingredients in mosquito repellents all over the world due to their low toxicity in animals, large doses cause neurotoxic symptoms such as tremors, loss of coordination, hyperactivity, paralysis, and an increase in body temperature. Other adverse effects include irritation of the skin and eyes, reproductive consequences, mutagenicity, immune system changes and so on. Recent research has also revealed that some pyrethroids may cause behavioural and developmental neurotoxicity, with particular concern for infants and children due to their potential exposure during a sensitive neurodevelopment stage. Pyrethroid insecticides have been shown in recent years to diminish sperm count and motility, cause sperm head deformity, increase the number of defective

sperm, damage sperm DNA, induce anueploidy, influence sex hormones levels, and cause reproductive toxicity (Fathy*et al.*, 2017).

2.6.NATURAL –PLANTA BASED MOSQUITO REPELLENT

People as well as scientific and technological breakthroughs, have long drawn inspiration from nature. Plant extracts have recently received a lot of interest from researchers all over the world. Natural repellents come from Asteraceae, Cupresseaceae, Labiatae, Laminaceae, Lauraceae, Meliaceae,Myrtaceae, Piperaceae, Poaceae, Rutaceae, Umbelliferae, and Zingiberaceae families. They have been tested for mosquito repellency against a variety of vectors, but only a few have been commercialised (Pridgeon *et al.*, 2017).

2.6.1.Use of plants as mosquito repellent

Fumigation, burning green leaves on the hut's threshold mosquito coil, pesticides, sprays and repellents are all common ways to avoid the deadly disease transmitted by mosquito bites. Smoke, for example, is widely used means of repelling biting mosquitoes all throughout the world. Fresh or dried herbs are frequently added to flames to improve the smoke's repelling qualities. Also, man has been exploiting the repellency of plants for thousands of years, simply by hanging bruised plants in buildings, a practice that is widely used in developing nations. As a result, using home grown repellents may lessen the demand for foreign imports, which can be costly due to exchange rate inequities and transportation costs. Plants have also been employed as crude fumigants, in which plants were burned to keep mosquitoes at bay, and later as oil formulation applied to the skin or clothing. Because many noxious smoke, it was supposed that they would repel insect (Moore *et al.*,2018).

Mosquito coils built from dried plant materials like saw dust are another inexpensive and effective mosquito repellent are usually culturally acceptable and well-known, in addition to being inexpensive and readily available. Steam distillation is used to extract essential oils from a variety of plants. Basil *(Ocimum basilicum*), mint (Mentha spp), hyptis *(Hyptis suaveolens),* and lavender *(Lavendula angustifolia)* are all members of the Lamiaceae family of plants, thyme (Thymus vulgaris.), and sage (Salvia spp.). Myrtaceae is a family of plants that includes a number of different species. *Eucalyptus* (*Eucalyptus* spp.) and *Melaleuca* (Melaleuca spp.). Citronella, lemongrass, and Palmarosa are all of the poaceae family. *Cymbopogo*n spp. are genus of Cymbopogon plants (Wendimu *et al.*, 2017).

2.7.PLANT SOURCE SELECTD – *Eucalyptus globules*

Eucalyptus globulus is a tree that normally grows to a height of 45 m (145ft), but can occasionally simply be a stunned shrub, or can grow as tall as 90-100 m (300-330 ft) under optimal conditions, and creates a lignotuber. The bark is normally smooth and white to cream coloured, but slabs of persistent, unshed bark can occasionally be seen at the base. Young plants and coppice regrowth have stems that are more or less square in cross-section with a pronounced wing on each corner, and are often several metres tall. Juvenile leaves re sessile, glaucous elliptic to egg shaped, and up to 150 mm and long and 105 mm wide, and are generally paired in opposing pairs. Adult leaves are the same glossy to dark green colour.

Many plants have proven to be effective and effective and environmentally friendly mosquito repellents, as they contain compounds with unique larvicidal action. The eucalyptus spp. leaves are high in the chemical cineole, which has great larvicidal properties and anti- mosquito-vector repelling qualities (Siashpoosh *et al.,* 2017).

Fuigure.5:*Eucalyptus globulus*

Other plants sources used along with Eucalpyptus globules includes powdered forms*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 induca* (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), *Vitex negundo*(Nochi),*Leucas aspera* (Thumbai), *Tinospora cordifolia* (Seendhal), *Eclipta prostrate* (Karisalanganni), *Solanum procumbens* (Thudhuvalai), *Cynodon dactylon* (Arugampul), *Cinnamomum camphora* (Camphor), *Shorea robusta* (Kungliyam).

2.8.ASSAYS TO RECORD THE EFFICACY OF INCENSE BAR

2.8.1. CAGE TEST

The mosquitoes utilised in the experiment must be pathogen-free according to WHO guideline for efficacy repellents, the cage measurement should be between 35-40 cm each side some studied have documented a cage dimension. Bano *et al*., employed a cage with diameter of 18×18×18 cm (Bano *et al., 2014)* while Phasomkusolil *et al* employed dimension of cage 30×30×30 cm (Phasomkusolil and Soonwera 2011), Anitha *et al* employed a 34×32×32 cm cage dimension, while chang *et al* reported cage size 35×35×35 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 baraccess that are likewise covered with netting. The cage must be filled with 50 mosquitoesthat have been deprived overnight and only fed sucrose solution according WHO. Then the *eucalyptus globules* computer incense bar were kept inside the cage and then the mosquitoes in the cage were exposed to smoke of computer incense bar for 45 minutes and the mortality data were recorded after every 15 minutes.

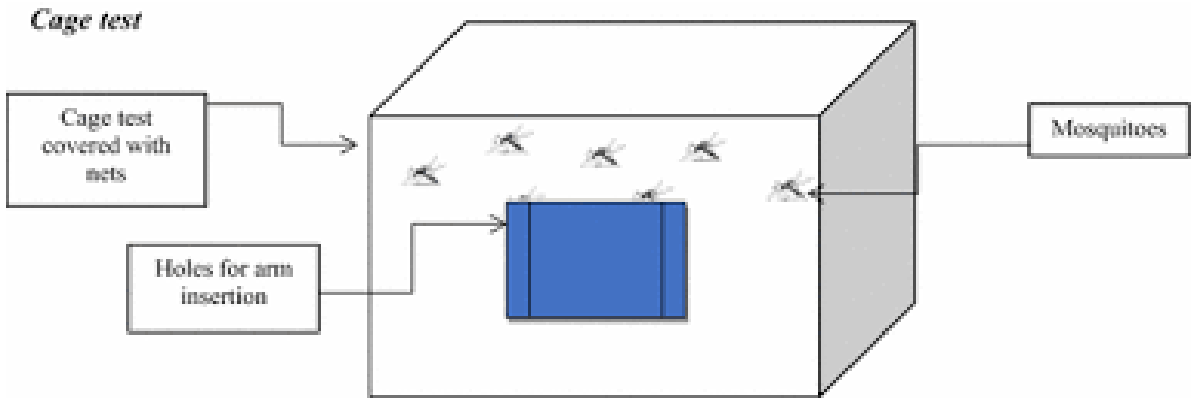


Figure 4: Cage test

Picture source:

https://www.google.com/imgres?imgurl=https%3A%2F%2Fmedia.springernature.com%2Flw685%2Fspringer-static%2Fimage%2Fart%253A10.1186%252Fs40691-016-0064-y%2FMediaObjects%2F40691_2016_64_Fig1_HTML.gif&imgrefurl=https%3A%2F%2Ffashionandtextiles.springeropen.com%2Farticles%2F10.1186%2Fs40691-016-0064-y&tbnid=Sey6TW6W-oVmsM&vet=12ahUKEwiFjfW3mtT3AhXagGMGHXmfDLQQMygBegUIARCrAQ..i&docid=4HVN4iLT_kNwBM&w=568&h=195&q=cage%20test%20for%20mosquito%20repellent&ved=2ahUKEwiFjfW3mtT3AhXagGMGHXmfDLQQMygBegUIARCrAQ

2.8.2.EXCITO CHAMBER

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 ad cone test method and cone test method does not involve the human subject to lure 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 over night 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 remaining mosquitoes inside the chamber which is 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 repellency was calculated using the formula (Aurfa *et al., 2016)*

% Mosquito repellency: (NES–NDE) (NEX)× 100

Where, NES corresponds to the number of mosquitoes escaped,

NDE refer to the number of mosquitoes dead

NEX represents the number of mosquitoes exposed

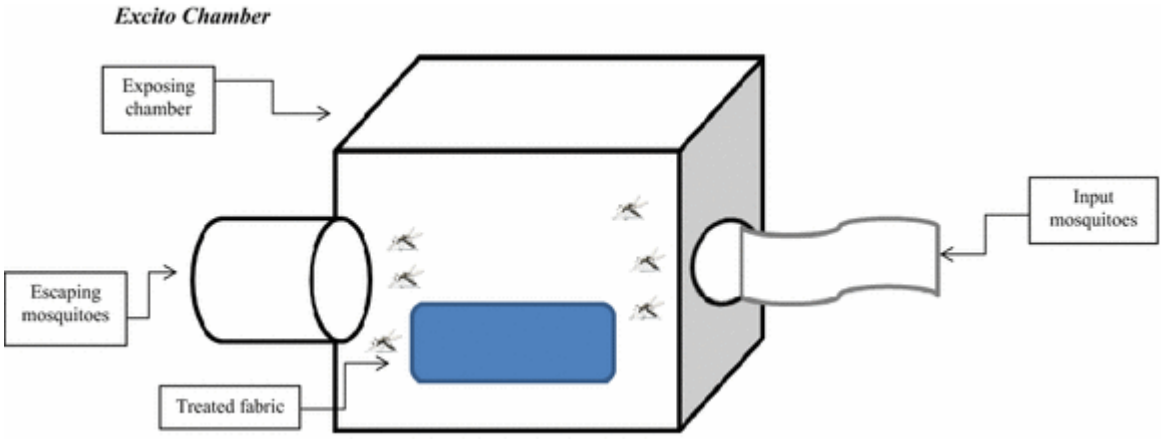


Figure5: Excito chamber test

Picture source:

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

Many of the vector borne disease that harms humans and other animals are transmitted by mosquitoes. Mosquitoes constitute a major public health problem as vectors of serious human disease. Immediate action is must to control the mortality and morbidity rate minimum, this can be done by insects repellents which is chemical or organic substances. 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 repellency is given in the following chapter.

METHODOLOGY

3. METHODOLOGY

The present study is to look in to the mosquito repellent activity of the incense bar made by *Eucalyptus globulus* against Aedes Spp. and Culex Spp.

3.1. PLANT COLLECTION

Fully developed fresh leaves of *Eucalyptus globulus* were collected from local area of Gudalur, Nilgiris. 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 LARVEA COLLECTION

Mosquito larvae and pupae of *Aedes* Spp. and *Culex* Spp. were collectedfrom National Centre for Disease control, (NCDC) Mettupalayam and 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 favorable 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 aqueous extract:

The plant sample was collected from local area of Gudalur, Nilgiris. 5g of fresh leaves 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 local area of Gudalur, Nilgiris. 5g of fresh leaves were collected, cleaned, grinded and extracted using ethanol. The extract was evaporated to dryness at 60℃ and concentrations ranging from 20mg were dissolved in 5µl of Dimethyl sulfoxide. Similarly, the plant extract was prepared using ethyl acetate and chloroform.

3.3.1.Biological assay:

Two different concentrations of 20 µg/ml and 40 µg/ml of aqueous extract,ethanolic extract, chloroform extract and ethyl acetate extracts were prepared in distilled water and tested for larvicidal activity of *Aedes* Spp. and *Culex* Spp. All experimental exposures were made in Petri-plate. Fifteen (15) larvae were collected with a Pasteur pipette and placed in a Petri plates containing two different test sample. 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 *Eucalyptus globulus* was checked and mortality was recorded.

Percentage mortality of larvae is calculated by,

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

C-Number of larvae survived in the control

T-Number of larvae survived in the test

3.4. Prepartion of mosquito incense bar:

3.4.1. Prepeparation of mosquito incense bar paste:

[A].Herbal base incense bar:

Powdered form 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), *Vitex negundo*(nochi), *Leucas aspera* (thumbai), *Tinospora cordifolia* (Seendhal), *Eclipta prostrate* (karisalanganni), *Solanum procumbens* (Thudhuvalai), *Cynodon dactylon* (Arugampul), *Cinnamomum camphora* (Camphor), *Shorea robusta* (Kungliyam) were mixed together.

[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, milk; the two derived products are curd and ghee. And jaggery is used as the binding agent in making computer incense bar.

[C].*Eucalyptus globulus,* herbal base and panchkavyam incense bar prepareation:

Eucalyptus globulus leaves were collected from local area of gudalur, Nilgiris. They were washed and cut into small pieces and dried in s hade for about 2 days. The dried parts were pounded and powdered using domestic grinder and mixed with herbal base, panchakavyam to make computer incense bar.

3.4.2.MOULDING OF MOSQUITO PASTE INTO INCENSE BAR

The mould was formed with a length of 66 cm and width of 7cm 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 6.

Figure 7.

Figure 8.

Figure 6-Herbal base incense bar

Figure 7-Herbal base and panchakavyam incense bar

Figure 8-Herbal base, panchakavyam and *Eucalyptus globulus* incense bar

3.4.3. MOSQUITO 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 baraccess that are likewise covered with netting. The cage must be filled with 50 mosquitoes that have been deprived overnight and only fed sucrose solution according WHO. Then the *eucalyptus globules* computer incense bar were kept inside the cage and then the mosquitoes in the cage were exposed to smoke of computer incense bar for 45 minutes and the mortality data were recorded after every 15 minutes.

EXCITO CHAMBER

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 ad cone test method and cone test method does not involve the human subject to lure 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 incense bar prepared with panchakavyam and *Eucalyptus globulus* is placed inside the chamber. The mosquito was starved over night 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 remaining mosquitoes inside the chamber which is 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 repellency was calculated using the formula (Adeela*et al., 2016)*

% Mosquito repellency: (NES –NDE) (NEX)× 100

Where, NES corresponds to the number of mosquitoes escaped,

NDE refer to the number of mosquitoes dead

NEX represents the number of mosquitoes exposed

3.4.4. COMPARATIVE STUDIES

Commercially available mosquito repellent, Good night and Maxo were checked for their repellency and compared with computer incense bar made with *Eucalyptus globulus* by 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 50 mosquitoes that have been deprived overnight and only fed sucrose solution according WHO. Then the E*ucalyptus globules* computer incense bar were kept inside the cage and then the mosquitoes in the cage were exposed to smoke of computer incense bar for 45 minutes and the mortality data were recorded after every 15 minutes (Aurfa *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 *Eucalyptus* Spp.are of prolific occurrence and about 15 species of them have been chemically examined. The chemical constituents and their biological activity of *Eucalyptus globulus* 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 *Eucalyptus globulus,* 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 *Eucalyptus globulus* leaves and mosquito repellent activity of incense bar made by using *Eucalyptus globulus* leaves and the result obtained are presented in this chapter.

4.1. Larvicidal activity:

Table: 4.1.1 - Larvicidal activity of Aqueous, Ethanol, Chloroform and Ethyl acetate extract of *Eucalyptus globulus* against *Aedes* Spp.

Extract	Concentration (µg/ml)	NO of larvae		Time duration	Percentage mortality
		Exposed	survived		
Distilled water (control)	-	15	0	24	0
Aqueous Extract	20	15	12	24	20
	40	15	10	24	33
Ethanol	20	15	6	24	60
	40	15	5	24	66
Chloroform	20	15	9	24	40
	40	15	8	24	46
Ethyl acetate	20	15	7	24	53
	40	15	6	24	60

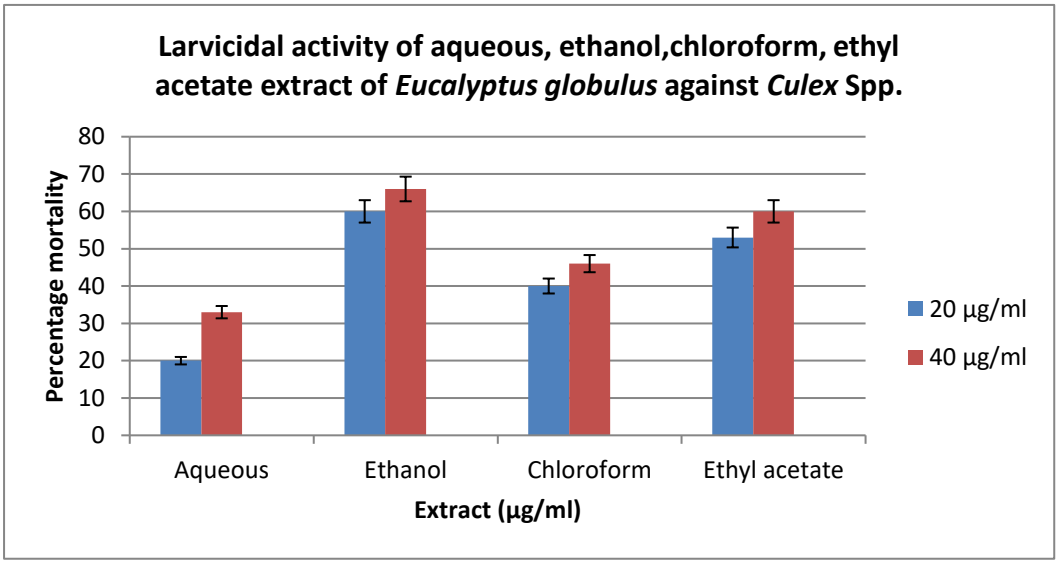
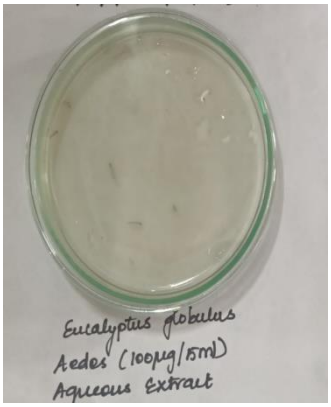


Figure.9: Graphical representation of Larvicidal acitivity of aqueous, ethanol, chloroform, ethyl acetate extract of *Eucalyptus globulus* against *Aedes* Spp.



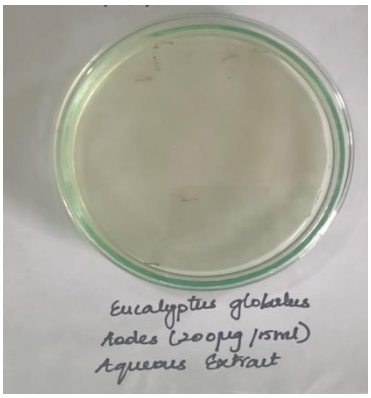
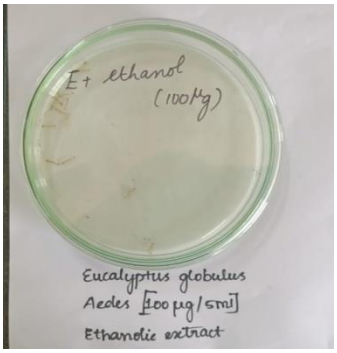
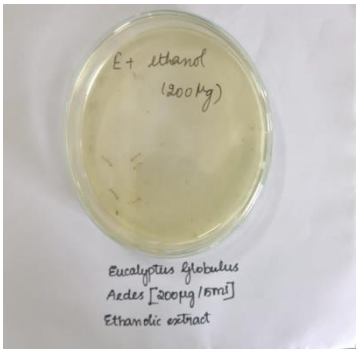
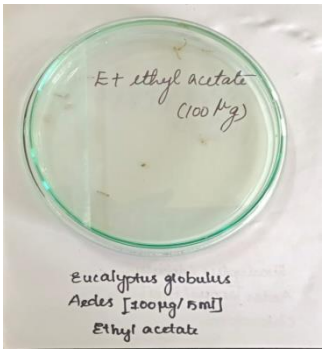


Figure 10. a. Distilled water b. Aqueous extract c. Aqueous extract

20 µg/ml 40 µg/ml

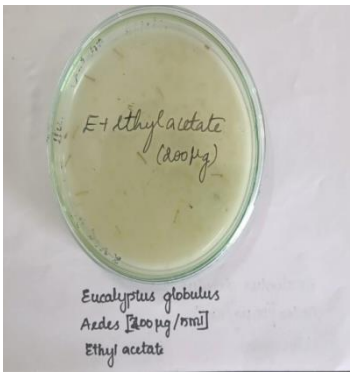


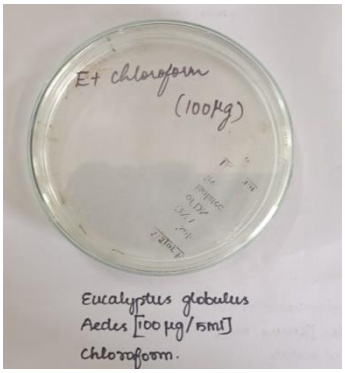


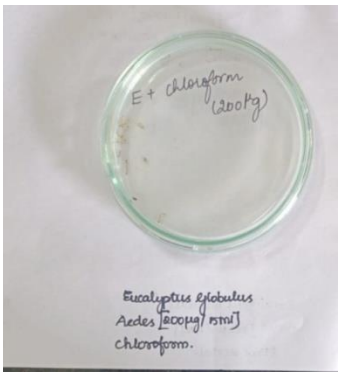


d. Ethanolic extract e. Ethanolic extract f. Ethyl actetate extract

20 µg/ml 40 µg/ml 20 µg/ml







g. Ethyl acetate extract h. Chloroform extract i. Chloroform extract

40 µg/ml 20 µg/ml 40 µg/ml

Figure 10. Larcvicidal activity of aqueous, ethanol, chloroform, ethyl acetate extract of *Eucalyptus globulus* against *Aedes* Spp.

Table 4.1.2. showed the larvicidal activity test of the leaf extract of *Eucalyptus globulus* in which ethanol extract showed the highest degree of mortality of 60 % (at 20 µg/ml) and 66 % (at 40 µg/ml) followed by ethyl acetate extract with the percentage of mortality of 53 % (at 20 µg/ml) and 60 % (at 40 µg/ml) then chloroform with the percentage of mortality of 40 % (at 20 µg/ml) and 46 % (at 40 µg/ml) while aqueous extract which showed the least degree of mortality 20 % (at 20 µg/ml) and 33 % (at 40 µg/ml).

Table: 4.1.2 - Larvicidal activityof Aqueous, ethanol, chloroform and ethyl acetate extract of *Eucalyptus globulus* against *Culex* Spp.

Extract	Concentration (µg/ml)	No. of larvae		Time duration	Percentage mortality
		Exposed	survived		
Distilled water (control)	-	15	0	24	0
Aqueous Extract	20	15	10	24	33
	40	15	9	24	40
Ethanol	20	15	5	24	66
	40	15	4	24	73
Chloroform	20	15	8	24	46
	40	15	7	24	53
Ethyl acetate	20	15	7	24	53
	40	15	5	24	66

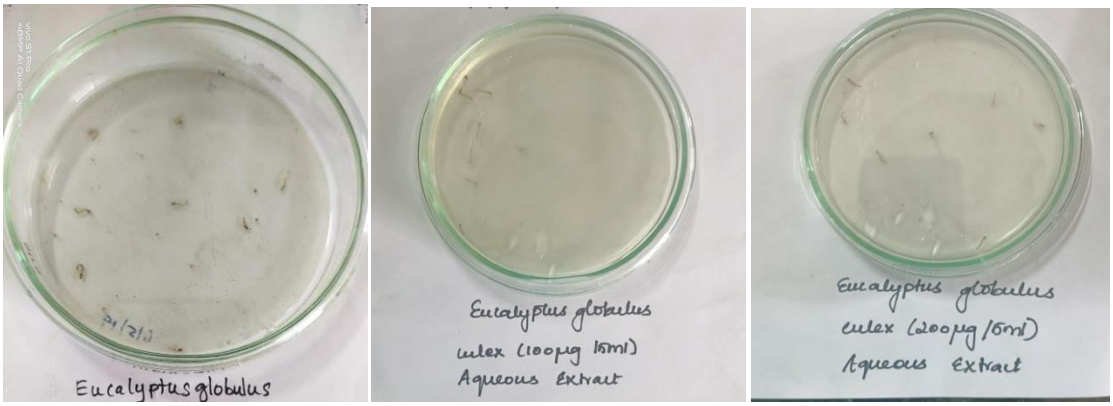
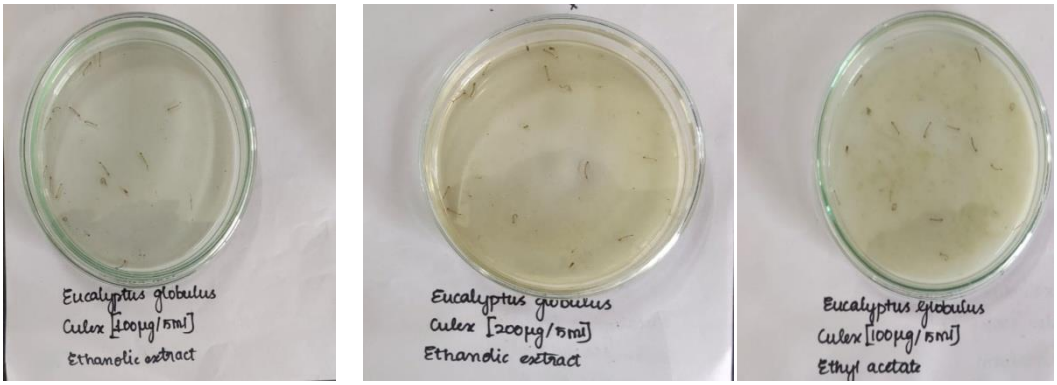


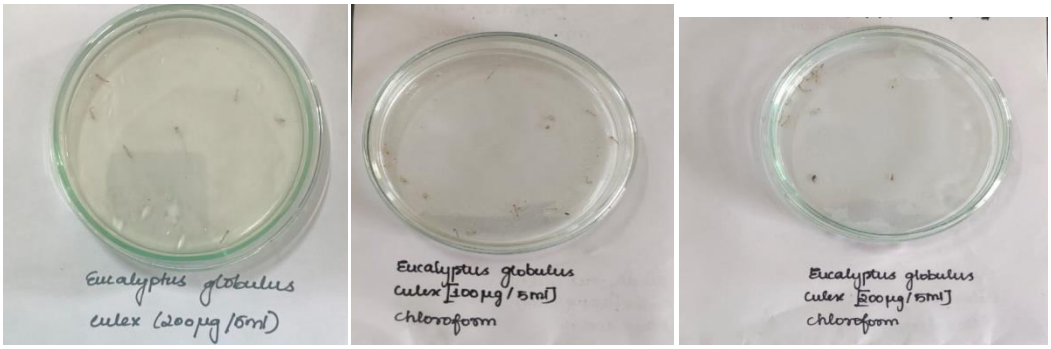
Figure.11. a. distilled water b. Aqueous extract c. Aqueous extract

20 µg/ml 40 µg/ml



d. Ethanolic extract e. Ethanolic extract f. Ethyl acetate

20 µg/ml 40 µg/ml 20 µg/ml



g. Ethyl acetate h. Chloroform i. Chloroform

40 µg/ml 20 µg/ml 40 µg/ml

Figure 11. Larvicidal activity of aqueous, ethanol, chloroform, ethyl acetate extract of *Eucalyptus globulus* against *culex* Spp.

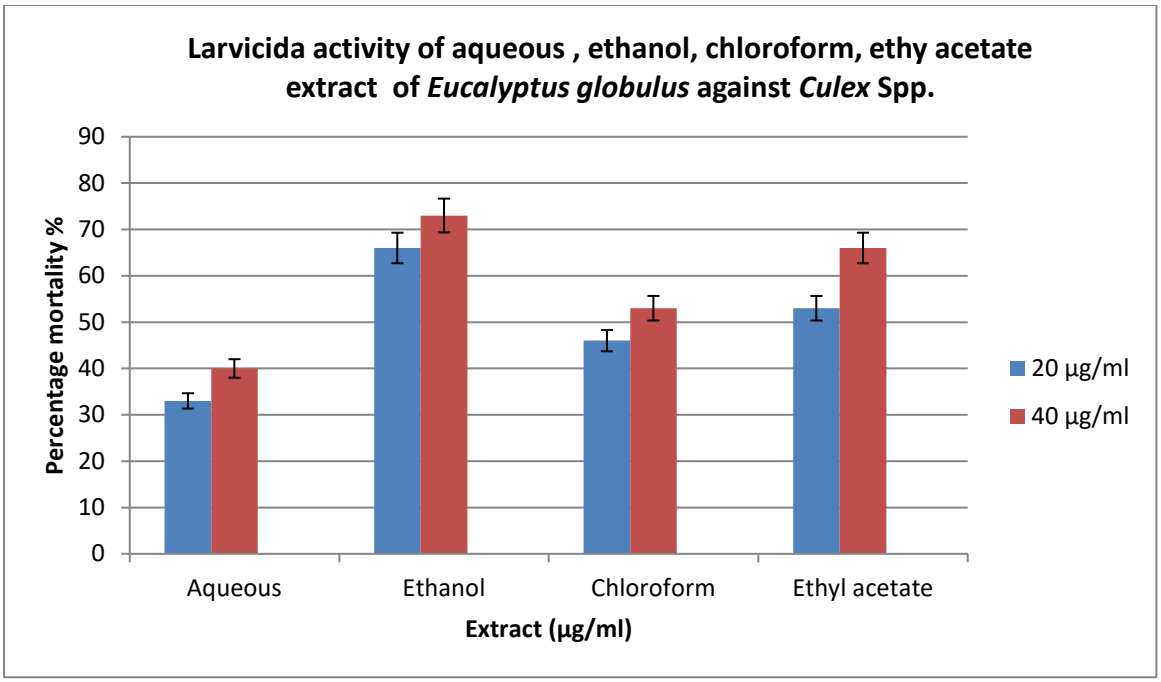


Figure.12. Graphical representation Larvicidal activity of aqueous, ethanol, chloroform, ethyl acetate extract against *culex* Spp.

Table 4.1.2. showed the larvicidal activity test of the leaf extract of *Eucalyptus globulus* in which ethanol extract showed the highest degree of mortality of 66 % (at 20 µg/ml) and 73 % (at 40 µg/ml) followed by ethyl acetate extract with the percentage of mortality of 53 % (at 20 µg/ml) and 66% (at 40 µg/ml) then chloroform with the percentage of mortality of 46 % (at 20 µg/ml) and 53 % (at 40 µg/ml) while aqueous extract which showed the least degree of mortality 33 % (at 20 µg/ml) and 40% (at 40 µg/ml).

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

In another study of larvicidal activity Teklani *et al.*, 2017 reprted that volume of 40.0 ml of distilled waterwas added into clean 100 ml beaker. Then to each beaker, eight mosquito larvae *(Aedes aegypti)* were added. A volume of 1 ml of plantextract 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 the present study, the larvicidal activity of Aedes and Culex was checked using extract prepared in aqueous, ethanol, chloroform, ethyl acetate extract at the rate of 20 µg/ml and 40 µg/ml. The ethanolic extract has shown highest mortality of 66% against *Aedes* Spp.similarly, 73% of mortality against *Culex* Spp.

4.2. Mosquito repellent activity:

Table 4.2.1. Efficacy of repellent in Cage test for *Aedes* Spp. mosquitoes

Incense bar Prepared using	**No. of Mosquito exposed**	**No. of Mosquito died after**		
		1 hour	**2 hours**	**3 hours**
Herbal base	20	0	2	3
Herbal base and panchakavyam.	20	2	4	6
Herbal base, panchakavyam and *Eucalyptus globulus* leaves powder.	20	5	5	7

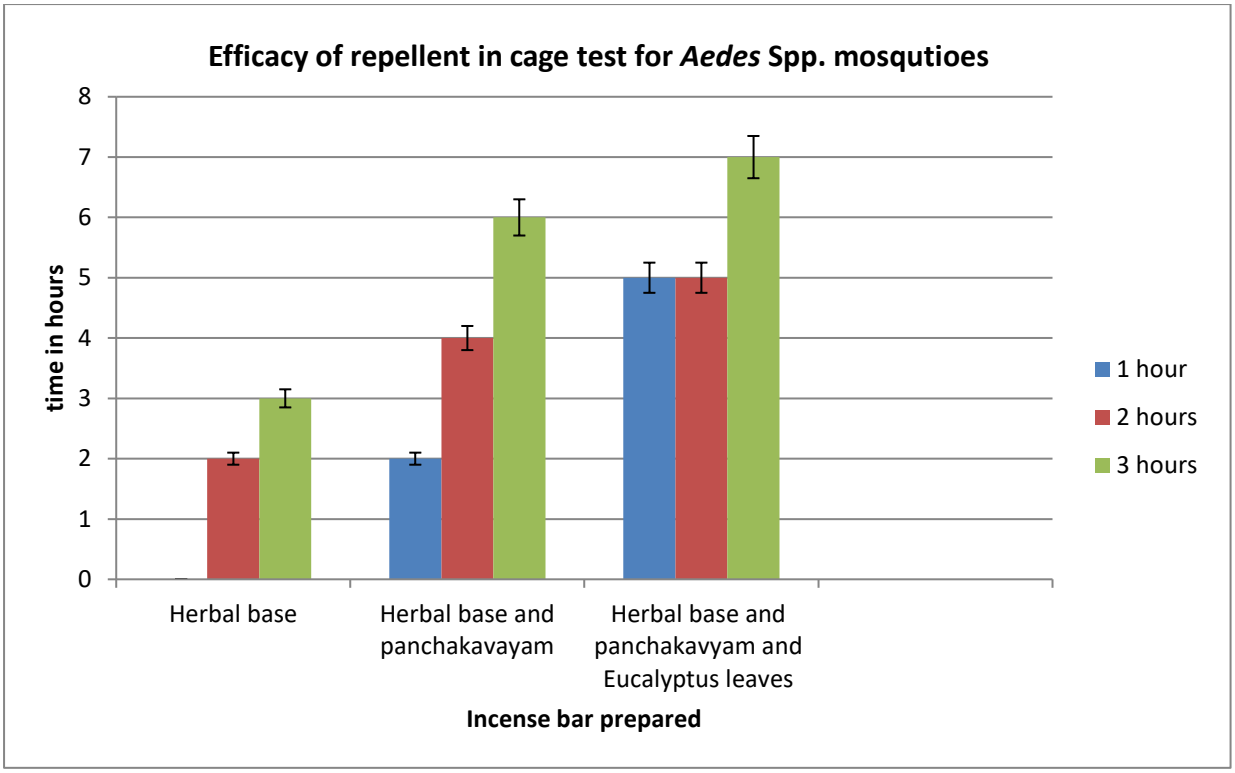


Figure 13.Graphical representation of efficacy of repellent in cage test for *Aedes* Spp. mosquitoes

Figure 14:Incense bar with Herbal base

Figure 15.Incense bar with herbal base panchakavyam

Figure 16: Incense bar with herbal base, panchakavyam along with *Eucalyptus globulus*

Table4.2.2.Efficacy of repellent in Cage test for *Culex* Spp. mosquitoes

Incense bar prepared using	No. of Mosquito exposed	No. of Mosquito died after		
		1 hours	2 hours	3 hours
Herbal base	20	0	4	6
Herbal base and panchakavyam.	20	3	6	9
Herbal base, panchakavyam and *Eucalyptus globulus* leaves powder.	20	6	8	10

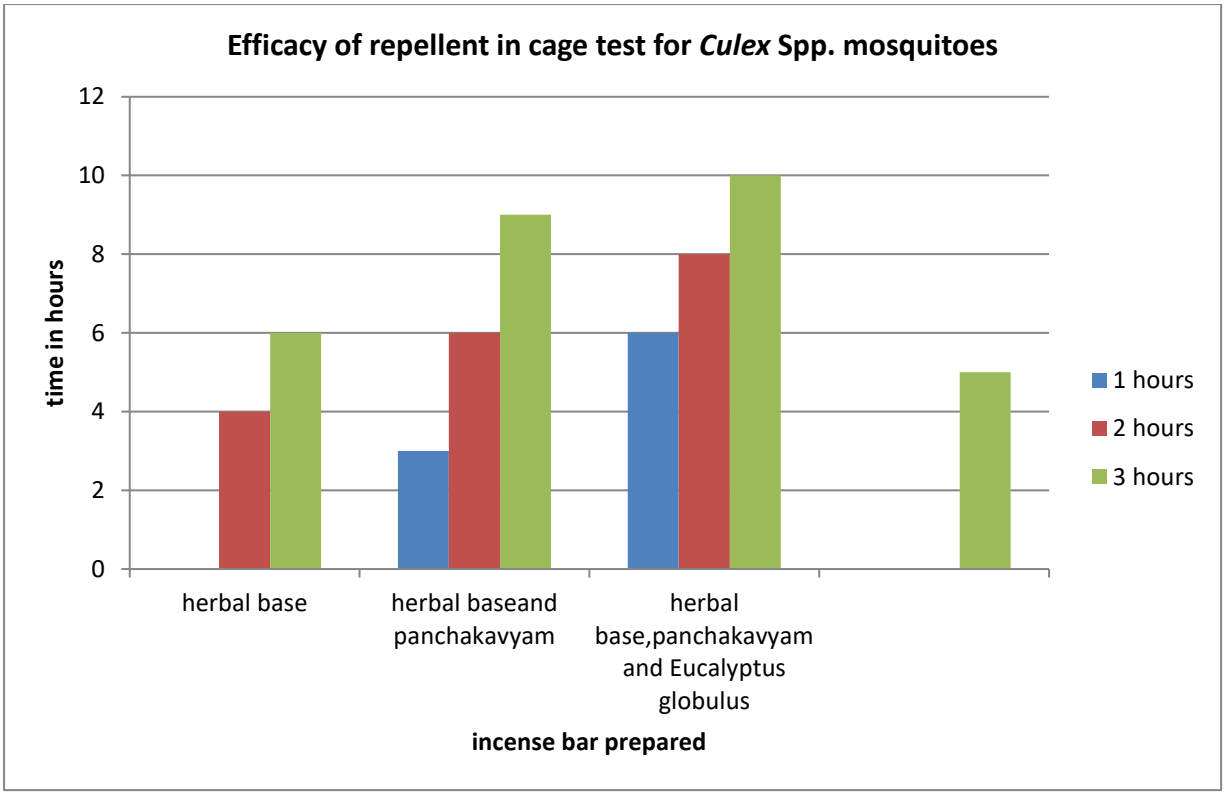


Figure 17.Graphical representation efficacy of repellent in cage test for *culex* Spp. mosquitoes

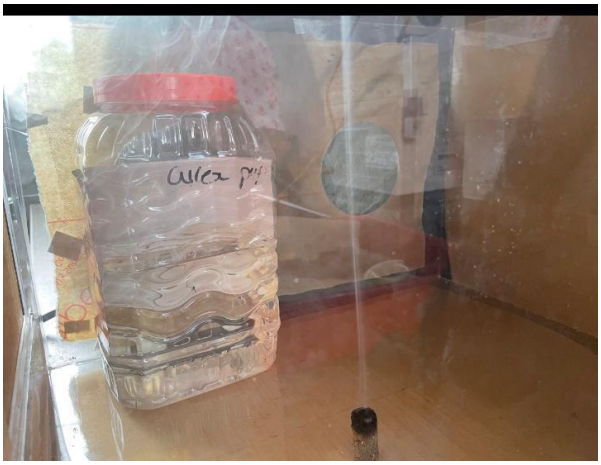


Figure 18: Incense bar with herbal base

Figure 19: Incense bar with herbal base and panchakavyam

Figure 20: Incense bar with herbal base, panchakavyam

along with *Eucalyptus globbulus*

Ponkiya *et al*.,2018 reported 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.

Shekhar *et al.,* 2014 evaluated that *Eucalyptus globulus* displayed the highest susceptibility to the hexane extract followed by the diethyl ether extract among *Anopheles stephensi,* on the other hand, showed the highest larval mortality in the dichloromethane extract followed by the hexane extract. The highest level of tolerance was observed in the strain of *Aedes aegypti* towards the methanol extract of *Eucalyptus globulus*. The above-cited studies undoubtedly indicated that *E.globulus* plants possess promising potent repelling activity against mosquitoes and other bloodsucking insect.

Loganet *et al.*, 2010 reported that *Anopheles gambiae s.s.* 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 with herbal products and panchakavyam and the incense bar with herbal products, panchakavyam and *Eucalyptus globulus* leaves kill 3, 6, 7 *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 *Eucalyptus globulus* leaves kill 6, 9, 10 *Culex* Spp. mosquitoes in 3 hours respectively. With these observation, we can say that *Eucalyptus globulus* plant displays mosquito repellent activity and it can be utilized to produce ecofriendly and cost effective mosquito repellent products in the form of incense bar.

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

Time (hours)	NEX	NES	NDE	Mosquito repellency %
1	20	5	5	50 %
2	20	6	6	60%
3	20	7	6	65%

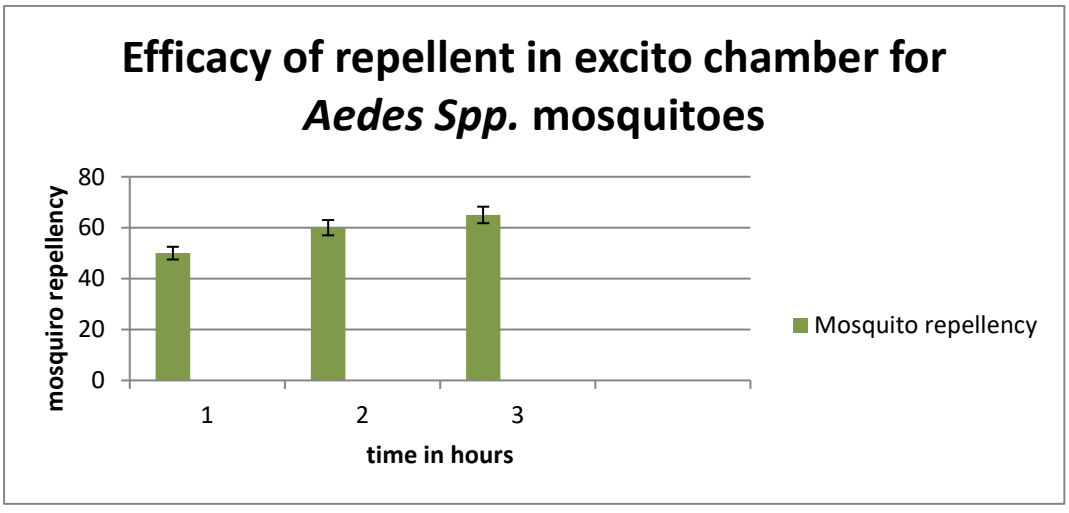


Figure 21. Graphical representation of efficacy of repellent in excito chamber for *Aedes* Spp. mosquitoes

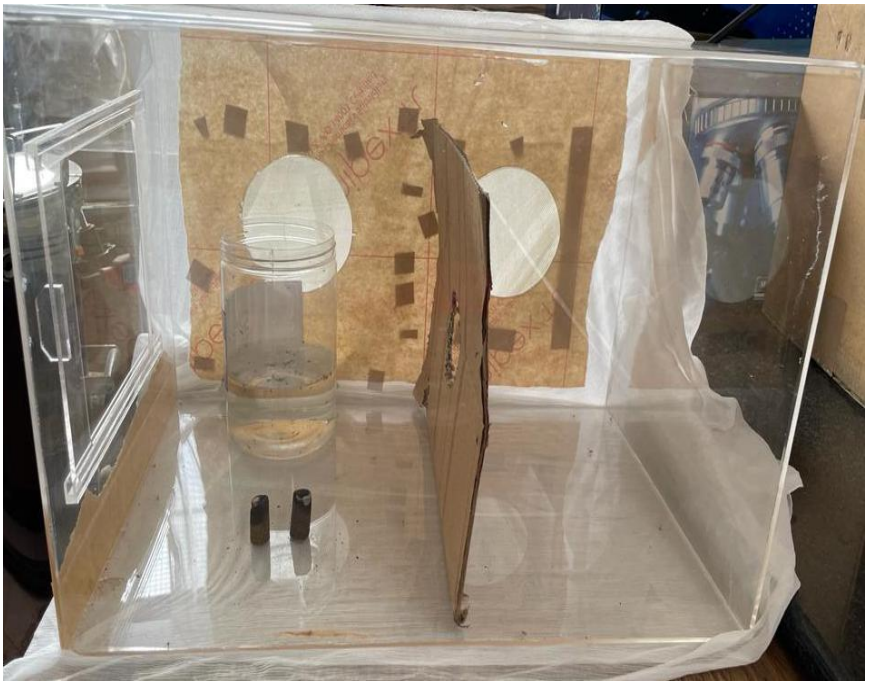


Figure 22. Efficacy of repellent in excito chamber for *Aedes* Spp. mosquitoes

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

Time (hours)	NEX	NES	NDE	Mosquito repellency %
1	20	5	4	50%
2	20	7	4	55%
3	20	8	4	60%

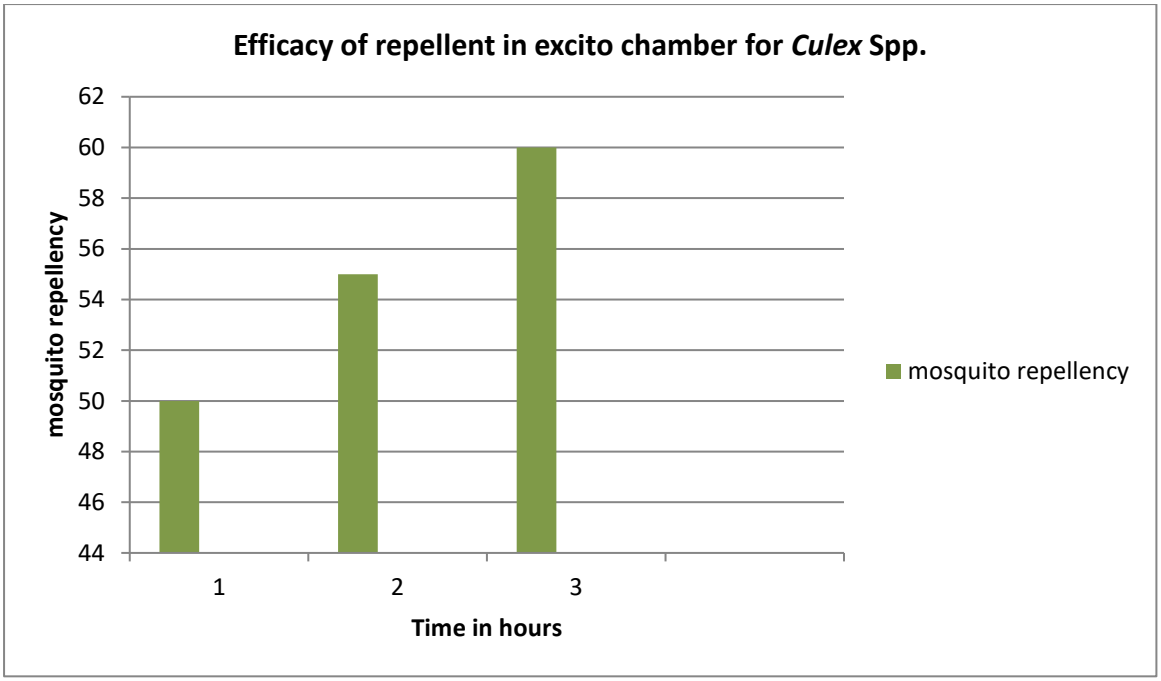


Figure 23. Graphical representation of efficacy of repellent in excito-chamber for Culex Spp. mosquitoes

Figure 24: Efficacy of repellent in excito chamber 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.,* 2008reported that 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 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 *Eucalyptus globulus* for the control of vector mosquitoes. Plants can provide safer alternatives for modern deadly poisonous synthetic chemical.

The present study indicates that incense bar using herbal products, panchakavyam and*Eucalyptus globulus* leaves show 50 % repellency in 1 hour, 60 % repellency in 2 hours 65 % and repellency in 3 hours against *Aedes* Spp. The incense bar using herbal products, panchakavyam and *Eucalyptus globulus* leaves show 50 % repellency in 1 hour, 55 % repellency in 2 hours and 60 % repellency in 3 hours against *Culex* Spp.

4.2.3 Comparative studies:

In this study we compare the commercially available mosquito repellent coil with the computer incense bar of *Eucalyptus globulus* for mortality rate by 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 *Eucalyptus globulus* 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).

Mosquito coil	Mosquito exposed	Mosquito dead
Goodnight	**20**	**20**
Maxo	**20**	**20**
***Eucalyptus globulus* computer incense Bar**	**20**	**13**

Table 4.2.5.comparative studies for commercially available mosquito coil and *Eucalyptus globulus* computer incense bar:

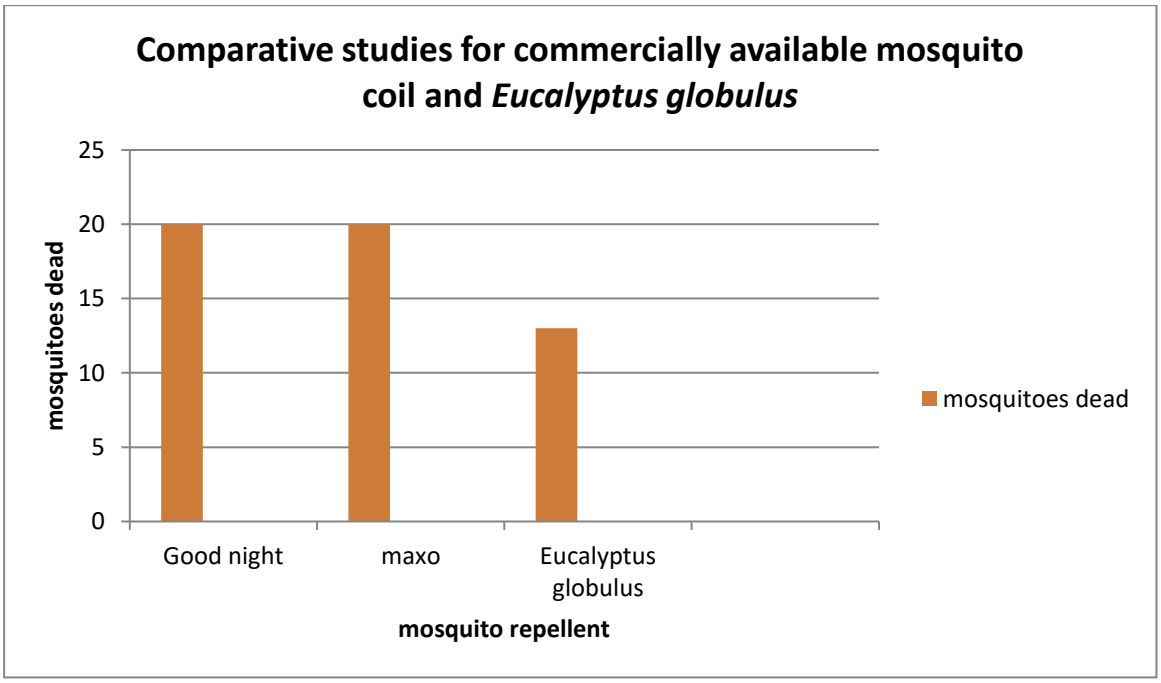


Figure 25. Graphical representayion of comparative studies for commercially available mosquito coil and *Eucalyptus globulus* computer incense bar

Figure 26: Maxo mosquito repellent activity by cage test

Figure 27: Good night mosquito repellent activity by cage test

Karunamoorthi *et al.,* 2016 reported that herbal pesticides gained importance recently; plant products are considerably safer and ecofriendly 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 ecofriendly biological materials for control of insect pests.

Madhubabu *et al.*,2012 in rats exposed to allethrin-based mosquito coil smoke for 15–180 days, compared to the unexposed controls, lipid peroxidation was increased in the caudal

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 reveealed that in 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 increasein 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.

In the recent years the emphasis to control the mosquito populations has shifted from the use of conventional chemicals towards more specific and environmental friendly materials, which are generally of botanical origin .*E. globulus* contains many phytochemicals and thus can act as new candidates for safe and effective alternate to chemical insecticides.

This study reveals that computer incense bar equally kill the mosquitoes of the commercially available mosquito coil. In the present investigation, we have identified ecofriendly substances leaves of *Eucalyptus globulus* 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 nonhazardous botanicals as insect repellent to target organisms. The current research that uses Cymbopogon citratus and Eucalyptus globulus has showed that plant in our environment can be harnessed and used for mosquito repellent. The present study is in agreement with the work of (Umar *et al.*,2021) who reported that plant material i.e Lowsoniainermis can be used as mosquito repellent using different extracts resulted in high percentage repellent of mosquito which shows high degree of efficacy against mosquito.

The leaf extracts of Eucalyptus globulus and *Centella asiatica* showed an overall moderate larvicidal effect when tested against different strains of *Ae. aegypti* and *An. stephensi*. Among the four extracts studied, the hexane extracts of both plants and the diethyl ether extract of *C. asiatica* demonstrated higher potential for the control of strains of mosquito vectors, followed by dichloromethane.

With this background the present study was designed, to check the larvicidal activity on larvae using the various extract of *Eucalyptus globulus.* 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 *Eucalyptus globulus* and compared their efficacy by performing two test

- 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 extaract shown high mortality rate against *Aedes* Spp. and *Culex* Spp.of 66% and 73% at 40 µg/ml respectively when it is compared with the other extracts like aqueous, chloroform and ethyl acetate extract.

Repellency test for Aedes 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 *Eucxalyptrus leaves* kills 3,6,7 mosquitoes in 3 hours respectively. Similarly, for Culex mosquito species the incense bar with herbal base, incense bar with herbal base and panchakavyam and incense bar with herbal base, panchakavyam along with *Eucalyptus globuylus* kills 6,9,10 mosquitoes in 3 hours respectively. From this observation we can say that *Eucalyptus globulus* 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 *Eucalyptus globulus* leaves show 50% repellency in 1 hour, 60% repellency in 2 hours and 65% repellency in 3 hours against *Aedes* Spp.The incense bar using herbal products, panchakavyam and *Eucalyptus globulus* leaves show 50% repellency in 1 hour, 55% repellency in 2 hours and 60% repellency in 3 hours against *Culex* Spp.

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 has which was made using *Eucalyptus globulus* extract 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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