STUDY ON MOSQUITO REPELLENT ACTIVITIES OF *CYMBOPOGON CITRATUS* AND ITS HERBAL COMPUTER INCENSE BAR

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(20PBT013)

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A Thesis submitted in

Partial fulfilment of the

Degree of Master of Science in Biotechnology

Avinashilingam Institute for Home Science and Higher Education for Women

Coimbatore – 641043

May 2022

CERTIFICATE

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INTRODUCTION

1. INTRODUCTION

The expansion of mosquito-borne diseases such as dengue, yellow fever, and chikungunya in the past 15 years has ignited the need for active surveillance of common and neglected mosquito-borne infectious diseases. Mosquitoes are significant vectors of human and animal illnesses, thus understanding their biodiversity and distribution in the Afrotropical region is critical for developing evidence-based vector control measures. *Anophele spp, Culex spp, Aedes spp, Eretmapodites spp, Mansonia spp, and Coquillettidia spp* are among the mosquito species implicated in the spread of infections in Cameroon. There were also reports of 26 human and zoonotic arboviral infections, one helminthic disease, and two protozoal diseases (Bamou *et al*., 2021).

Mosquitoes spread some of humanity's most serious infectious diseases, such as malaria, which kills between 0.6 and 1.2 million people each year, the majority of whom are children in low-income nations. It is becoming increasingly clear that no one action will be sufficient to eradicate malaria, and that a multifaceted approach, including vector control, is required. There are currently very efficient vector control strategies available, the most of them use insecticides, though there is evidence of resistance spreading. A number of innovative genetic techniques to vector control are now being tested. Massive investments in molecular resources, especially the distribution of several full-genome sequences, have tremendously aided mosquito research. Vector management is population biology in action, and I believe that continued success will necessitate paying as much attention to mosquito ecology as mosquito molecular biology (Charles *et al*., 2012).

Insecticidal activity of plant-based products against mosquitoes has been the subject of extensive investigation in recent decades. This is a modern and timely parasitology challenge aimed at reducing the widespread usage of synthetic pesticides, which is increasing mosquito resistance and posing major health and environmental risks. This study examines the vast amount of research on plant extracts used as mosquito larvicides, notably aqueous and alcoholic extracts, which are easy to formulate in water without the need of surfactants. We analysed data from over 400 plant species and discovered that 29 of them show exceptional larvicidal activity (LC50 values below 10 ppm) against key vectors such as *Anopheles spp, Aedes spp and Culex spp* among others. In addition, the synergistic and antagonistic effects of plant extracts and conventional pesticides, as well as among specific plant extracts, are explored. The efficacy of pure chemicals obtained from the most effective plant extracts, as well as their mechanism of action and non-target species impact, are all discussed. Alkaloids, alkamides, sesquiterpenes, triterpenes, sterols, flavonoids, coumarins, anthraquinones, xanthones, acetogenins, and aliphatics are examples of secondary metabolites. On mosquito larvae, their route of action spans from neurotoxic effects to detoxification enzyme inhibition, larval growth, and/or midgut damage (Roman *et al*., 2019).

The insect repellant N,N-diethyl-m-toluamide (DEET) is considered in terms of biodistribution and toxicity. Workers who use repellant containing this component may be exposed to more than 442 g during the course of six months. Variable penetration into the skin of 9 to 56 percent of a topically administered dose has been recorded in human investigations, with roughly 17 percent absorption into the circulatory system. Humans excreted DEET more quickly than animal models, but not as completely. Humans eliminated just around half of the ingested DEET after 5 days. DEET depot storage in the skin has also been demonstrated. There have been reports of skin irritation, including scarring bullous dermatitis in humans. Other researchers were unable to validate one animal study that claimed embryotoxicity. Limited mutagenicity and carcinogenicity tests yielded negative results. Workers exposed to 4 g or more per week showed neurotoxic consequences. After being exposed to various levels of DEET ranging from minor to enormous doses, six young girls suffered encephalopathies. Three of the girls died subsequently. The cause of their deaths is yet unknown. More research into absorption, carcinogenicity, and neurotoxic effects is needed due to a paucity of information (Robbins *et al*., 1986).

Chemical components like as N, N-diethyl-metatoluamide (DEET), Allethrin, N, N-diethyl mendelic acid amide, and Dimethyl phthalate are used in the majority of commercial repellents. Chemical repellents have been determined to be hazardous to public health and should be used with caution due to their negative effects on synthetic fabrics and plastics, as well as toxic reactions such as allergy, dermatitis, and cardiovascular and neurological side effects, which have been reported frequently following their misuse. The widespread use of chemically derived synthetic repellents for mosquito control has disrupted natural ecosystems, resulting in pesticide resistance, a return of mosquito populations, and negative effects on nontarget animals. As a result, adopting natural mosquito repellents as an alternative to developing new eco-friendly repellents could be a win-win approach for reducing negative effects on the environment and human health (Asadollahi *et al*., 2019).

Nature is an endless source of inspiration for both individual's 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 commercialized. 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 *A. gambiae*, implying that the kind of carbon where the hydroxyl substitution is present influences repellency (Khater *et al*., 2016).

Plants have traditionally been used to repel mosquitoes, and they are regarded to be more environmentally friendly than synthetic insecticides, which pose a significant risk to human health and the ecosystem. Researchers used several testing approaches to uncover repellent compounds efficient against mosquitoes, with the major focus of this review being essential oils and traditional botanicals. *Eucalyptus spp., Ocimum spp.,* and *Cymbopogon spp.* are the most commonly mentioned plant species. Furthermore, combining vanillin as a synergist and formulation techniques such as microencapsulation and nanoemulsion can improve the effectiveness of essential oils and their protection time (Rehman *et al*., 2014).

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

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

- To prepare the water extract, ethanolic extract, chloroform extract and ethyl acetate extract of *Cymbopogon citratus* and checking their larvicidal activity on the species of *Aedes spp and Culex spp*.
- To prepare computer incense bar with *Cymbopogon citratus* leaves, herbal products.
- To prepare panchakavyam and assessing the mosquito repellent activity against *Aedes spp* and *Culex spp*.
    1. Cage test
    2. Excito- chamber

- To compare the *Cymbopogon citratus* 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 vast literature is presented in the following chapter.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 MOSQUITO BORNE DISEASE:

Mosquito-borne diseases cause a huge global burden of disease and have posed a serious threat to humanity since the dawn of civilisation. Nearly 700 million individuals are infected with mosquito-borne diseases such as dengue, malaria each year. Although the bulk of these cases occur outside of the United States, more than 20 million Americans visit each year to areas where mosquito-borne illnesses are common. Further, it is anticipated that 50 million western travellers visit tropical regions of the world each year, where mosquito-borne diseases are frequent (Bagavan *et al*.,2011).

Infectious Plasmodium organisms can have zoonotic or human reservoirs, and they are generally found in tropical and subtropical areas. Patients with these infections may be in the early stages of a potentially life-threatening illness, which can lead to bad results if not detected by the clinician. Dengue and West Nile virus are the two mosquito-borne viral infections that have the greatest human impact (Caraballo *et al*., 2014).

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 vector- borne disease in the pacific, including those transmitted by mosquitoes. Malaria, Barmah Forest virus illness, and Ross River fever and these 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 area, 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 and chikungunya virus (CHIKV) (Walter *et al*., 2019).

2.1.2 **ZIKA VIRUS**

The Zika virus (ZIKV) is an arthropod-borne virus (arbovirus) that belongs to the Flaviviridae family and the genus Flavivirus. In 1947, ZIKV was isolated from a monkey in Uganda's Zika jungle. Chikungunya is a mosquito-borne disease caused by the chikungunya virus. ZIKV is spread by infected mosquito bites and has been isolated from a variety of Aedes mosquito species. including *Aedes aegypti*, which is found across the tropics and subtropics, and *Aedes albopictus*, which is found throughout Europe, particularly in Mediterranean countries. Aedes polynesiensis is thought to play a role in zika virus (ZIKV) transmission in FP (Musso *et al*., 2014).

2.1.3 DENGUE

Age, secondary infection, immunologic status, dengue serotype, and genotype all influence the clinical aspects of dengue disease in humans, which range from atypical non-severe or nonspecific febrile symptoms to potentially lethal dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). *Aedes aegypti* is the primary vector for dengue illness transmission, but *Aedes albopictus*, a less effective vector, has been surprisingly responsible for the disease's spread in recent years. 4 Migration to metropolitan areas, travel, and a lack of environmental control all contribute to the rise and rapid spread of these vectors on a global scale. As a result, Aedes spp. and the four dengue serotypes they carry are found in practically every tropical and subtropical region of the world, resulting in endemic and hyperendemic zones (Cucunawangsih *et al*., 2017).

2.1.4 MALARIA

Malaria is spread to humans by the bite of a female *Anopheles spp* mosquito carrying Plasmodium parasites. Plasmodium falciparum is the parasite that causes the most deaths of the five parasite species that infect humans. The most common sign of malaria is fever. Headache, cough, nausea, vomiting, abdominal pain, diarrhoea, and muscular or joint pain are all possible complications. In 2019, there were an estimated 229 million cases of malaria worldwide, with 409 000 deaths, with Sub-Saharan Africa having the highest number of cases and deaths. Each year, about 2000 cases of malaria are diagnosed in the United States, with the majority of cases contracted by US tourists who have visited malaria-endemic countries (Kristin *et al*., 2022)

2.1.5 YELLOW FEVER

Yellow Fever is a vector-borne disease caused by the yellow fever virus (YFV) being transmitted to a human through the bite of an infected mosquito. It is endemic to Sub-Saharan Africa and tropical South America, where it is thought to cause 200,000 clinical cases and 30,000 fatalities per year. Human infection can cause haemorrhagic fever, which is deadly in 20 percent to 50 percent of patients with severe disease. The majority of YFV infections in people are asymptomatic. The clinical signs of the disease range from a mild, nonspecific febrile illness to severe jaundice and haemorrhagic features. Symptomatic yellow fever (YF) infections usually begin with a rapid onset of fever and headache after a 3-6 day incubation period. Other symptoms such as photophobia, myalgias, arthalgias, epigastric discomfort, anorexia, vomiting, and jaundice may develop as the illness advances. Multisystem organ failure with haemorrhagic signs and symptoms occurs in some people (Staples *et al.,* 2010).

2.2.1. COMMERICALLY AVAILABLE MOSQUITO REPELLANTS

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

Repellents are classified in to five categories based on 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 block attraction to humans either by reducing the attractiveness of the host or a 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

Before World War II, MRs were mostly plant-based, with citronella oil being the most extensively used compound and the benchmark against which others were evaluated. Synthetic chemical repellents begin to appear around this time. Only three repellents were available: dimethylphthalate, which was found in 1929, Indalone (butyl-3,3-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate), which was patented in 1937, and Rutgers 612 (ethyl hexanediol), which was marketed in 1939. Later, 6-2-2 M-250 (a mixture of six parts DMP and two parts of Indalone and Rutgers 612) was utilised for military purposes. Because the Pacific and North African theatres offered considerable disease dangers to allied military personnel, World War II served as the principal catalyst for the development of novel repellent technologies. Between 1942 and 1947, almost 6000 compounds were examined in a range of research organisations, resulting in the discovery of numerous effective repellent chemistries. DEET, one of the most effective and extensively used insect repellents to date, was discovered as a result of multiple independent research studies. Several compounds have been created since then, based on prior studies that revealed amide and imide molecules as particularly effective contact repellents. Picaridin, a piperidine carboxylate ester, and IR3535 are two of them, and in some repellency bioassays, they are regarded DEET competitors (Khater *et al.,* 2017).

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 and has a 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 it has since become a staple insect and arthropod repellent. DEET is the most researched insect repellent 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 percent. The shorter protection time was also influenced by the combination. Because of its expensive cost, disagreeable odour and also there is a discomfort of continual application on exposed skin at high concentrations, DEET plays a limited role in disease control in the endemic areas (Leal *et al.,* 2014).

Figure: 1- N, N-diethyl-3-methylbenzamide

PERMETHRIN

Permethrin is a pyrethroid that may be used as both an insecticide and a repellent. Mosquitoes, flies, ticks, fleas, human lice, and chiggers are all susceptible to the toxin. It has a low toxicity in mammals and is poorly absorbed via the skin. Permethrin-containing products should be used on garments or other materials (tents, mosquito nets, etc.) rather than on the skin. Permethrin-treated clothing, when combined with DEET, creates a powerful barrier against biting insects and can almost completely eradicate mosquito bites. Permethrin yard foggers can be an efficient short-term treatment for modest outdoor settings, however, there have been some concerns expressed regarding the possibility of respiratory and gastrointestinal distress from continuous permethrin inhalation (Dill *et al.*, 2011).

Figure: 2 - Permethrin

Insect repellent 3535

In comparison to insect repellent 3535, a natural and pure synthetic repellent solution, learning from nature provided a molecule with exceptional effectiveness (IR3535). For the development of the topical IR 3535, scientists looked to nature for inspiration, with the goal of creating a molecule with optimised protection times and low toxicity. The base module was the naturally occurring amino acid -alanine, and the end groups were chosen to avoid toxicity while increasing efficacy. Merck IR3535 was developed in 1970 and is known as Merck IR3535. It has been accessible in Europe from 1970, but not in the United States until 1999. IR3535 is used to treat mosquitoes, ticks, flies, fleas, and lice in both humans and animals. It is also known as ethyl-N-acetyl-N-butyl-alaninate, ethyl butylacetylaminopropionate (EBAAP), β-alanine, and N-acetyl-N-butyl-ethyl ester. Its chemical formula is C11H21NO3. IR 3535's protection may be comparable to DEET's; however, it requires reapplication every 6–8 hours. IR3535 can be found in a variety of products, including Avon's Skin So Soft Bug Guard Plus Expedition. Although 20% IR 3535 gives perfect protection against *Aedes spp* and *Culex spp* mosquitos (up to 7–10 hours), it provides less protection against *Anopheles spp* (about 3.8 hours), limiting its use in malaria-endemic areas. Several field studies have found that IR 3535 is as effective as DEET in repelling mosquitoes of the Aedes and Culex genera but may be less effective in repelling anopheline mosquitoes; an uncontrolled field study of an IR 3535 controlled release formulation reported that these formulations may provide complete protection against mosquito biting for 7.1–10.3 hours (Murugan *et al.*, 2017).

ETHYL ANTHRANILATE

Ethyl anthranilate (EA) is a relatively novel entomological compound that has received a lot of interest in repellent research in recent years and is being evaluated as a better alternative to DEET. It is a nontoxic, volatile food additive that has been approved by the US Food and Drug Administration. EA is new and repellent against *Aedes aegypti, Anopheles stephensi, and Culex. quinquefasciatus,* with ED50 values of 0.96, 5.4, and 3.6 percent w/v, respectively, and CPTs of EA, 10 percent w/v of 60, 60, and 30 minutes, respectively, in the arm-in-cage method. Furthermore, its spatial repellency was found to be particularly effective against all three mosquito species tested. EA produced results that were comparable to the conventional repellent DEPA. As a result, EA's 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 (Abouelella *et al.,* 2018).

DEPA

DEPA (N,N-diethyl-2-phenyl-acetamide) is a repellent that was produced about the same time as DEET that repels a wide range of insects, although it never gained the same notoriety as DEET. DEPA has been shown to be nearly as effective as DEET in repelling mosquito vectors such as *Aedes aegypti, Aedes albopictus, Anopheles stephensi*, and *Culex quinquefasciatu*s. It has recently regained popularity and may prove to be a significant competitor to DEET, particularly in underdeveloped countries, because to its lower cost ($25.40 per kg vs. $48.40 per kg for DEET) (Marimuthu *et al.,* 2017).

2.3.1. NATURAL REPELLENT

Nature has long served as a source of inspiration for both humans and scientific and technological advancements. Recently, there has been lot pf 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 *et 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 i.e., 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).

2.3.2. OIL OF LEMON EUCALYPTUS

Mosquitoes, black flies, biting midges, ticks, and gnats are all attracted to the oil of lemon eucalyptus (p-menthane 3,8-diol or PMD), which is one of many plant-based insect repellents. Plant-based repellents are not as efficient as synthetics like DEET and Picaridin, although oil of lemon eucalyptus has been demonstrated to provide protection comparable to 20-30 percent DEET doses, but for considerably shorter lengths of time. Oil of lemon eucalyptus should not be used on children under the age of three, according to the product label, and it should be kept out of the eyes because it might cause substantial eye discomfort. (Dill *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) have 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: 3 - Lemon eucalyptus

2.3.3. CITRONELLA

Around 1858, the term "Citronella" was created from the French word "citronelle." It was first extracted for use in perfumery and later employed to repel mosquitoes by the Indian Army at the turn of the twentieth century, before being registered for commercial usage in the

United States in 1948. Citronella (5–10%) is one of the most extensively used natural repellents on the market today; these concentrations are lower than most other commercial repellents, and higher quantities can induce skin sensitivity. Citriodiol-containing products had the most efficient mosquito repellent profile among plant-derived compounds. EOs and extracts from plants in the Citronella genus (Poaceae) are commonly utilised as ingredients in plant-based mosquito repellents, particularly *Cymbopogon nardus*, which is sold commercially in Europe and North America. Citronella contains citronellal, citronellol, geraniol, citral -pinene, and limonene, which have a similar effect to DEET, but the oils evaporate quickly, causing efficacy to be lost and leaving the user unprotected. Citriodiol-containing products had the most efficient mosquito repellent profile among plant-derived compounds. Citronella-based repellents are not suggested for visitors going to disease-endemic areas, but if effective alternatives are too expensive or unavailable, using citronella to prevent mosquito bites may provide vital disease vector protection. Even though citronella-based repellents only provide protection from host-seeking mosquitoes for a limited period of time (2 hours), new formulations may be able to extend that duration (Khater *et al.,* 2017).

Figure: 4 - Citronella

2.3.4. NEEM

The limonoids in the neem tree have a wide range of activities against insect pests and microbiological diseases. The main active principles extracted from neem seed are complex tetranortriterpenoids such azadirachtin, salanin and nimbin. In comparison to synthetic pesticides, neem-based pesticidal formulations are widely regarded as organic and have been found to have low toxicity against non-target beneficial organisms. Azadirachtin, a biopesticide derived from neem seeds that is non-persistent in the environment, is one of the world's most successful biopesticides. Due to genetic variability and environmental variables, its composition varies greatly among trees in different areas (Sidhu *et al.,* 2003).

Figure: 5 - Neem

2.3.5. 2-UNDECANONE

Tick and mosquito repellent 2-undecanone (methyl nonyl ketone or IBI-246) is an oily, plant-based repellent that has demonstrated modest effectiveness. It is relatively recent to utilise 2-undecanone as an insect repellent; it is most typically used to repel cats, dogs, and even raccoons. Adverse effects of 2-undecanone as an insect repellent have not been thoroughly studied due to its limited use. According to studies, there is the possibility for some acute toxicity and moderate discomfort, but more research is needed (Dill *et al.,* 2011).

2.3.6. CATNIP (*NEPETA CATARIA*)

Catnip is a perennial plant in the Labiatae family of mints. This herb can be found throughout Central Europe, Central Asia, and the Iranian plateaus. Amer et al. found that a 20 percent oil solution of catnip provided 100 percent protection against *Anopheles stephensi* for 8 hours and was effective in preventing *Anopheles spp* mosquitoes. Nonetheless, Birkett et al. in Kenya found that the percentage repellency of catnip is dose dependent, with repellency percentages of 17 percent, 97 percent, and 100 percent, respectively, against *Anopheles gambiae* when using 0.01 mg, 0.1 mg, and 1 mg solutions of this herb (Asadollahi et al., 2019).

2.4.1. PLANT SOURCE

Cymbopogon citratus

TAXONOMIC CLASSIFICATION

- Kingdom - Plantae
- Phylum - Spermatophyta
- Subphylum - Angiospermae
- Class - Monocotyledonae
- Order - Cyperales
- Family - Poaceae
- Genus - Cymbopogon
- Species - *Cymbopogon citratus*

Figure: 6 - *Cymbopogon citratus*

Cymbopogon citratus is a short-rhizomatous perennial grass. Culms are tufted and strong, growing up to 2 m tall and 4 mm in diameter, with farinose nodes. Leaf blades glaucous, 30-90 x 0.5-2 cm, both surfaces scabrid, base gradually narrowing, apex long acuminate; ligule 1 mm; leaf sheaths glabrous, greenish inside; leaf sheaths glabrous, greenish inside; leaf blades glaucous, 30-90 x 0.5-2 cm, both surfaces scabrid, base gradually narrowing, apex long acuminate; ligul Spatheoles 1.5-2 cm; racemes 1.5-2 cm; rachis internodes and pedicels 2.5-4 mm, loosely villous on margins; pedicel of homogenous pair not swollen; spatheoles reddish or yellowish brown, 1.5-2 cm; racemes 1.5-2 cm; rachis internodes and pedicels 2.5-4 mm, loosely villous on margins; pedicel of homogenous pair not swollen Lower glume flat or slightly concave toward base, sharply 2-keeled, keels wingless, scabrid, veinless between keels; upper lemma narrow, entire and awnless, or slightly 2-lobed with 0.2 mm mucro; upper lemma narrow, entire and awnless, or slightly 2-lobed with 0.2 mm mucro. 4-5 mm pedicelled spikelet (Rojas *et al.,* 2016).

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 *Cymbopogon citratus* vs *Culex tritaeniorhynchus*, the disease’s vector Japanese encephalitis is a disease that affects the brain (Govindarajan *et al.,* 2008).

2.4.2. SELECTED HERBS

1. *CHRYSOPOGON ZIZANIOIDES* (VETIVER)

Vetiver is most closely related to Sorghum, although it has a lot in common with other aromatic grasses including lemongrass (*Cymbopogon citratus),* citronella (*Cymbopogon nardus, C. winterianus*), and palmarosa (*Cymbopogon martinii*). Vetiver oil has been used to track the mosquito populations in Sub-Saharan Africa during dry seasons. Mosquitoes were tagged with vetiver oil-soaked strings and then released. The marked mosquitoes were discovered in the areas like tree holes and ancient termite mounds by dogs trained to track the scent (Sohn *et al.,* 2014).

2. *NARDOSTACHYS JATAMANSI* (JADA MANJI)

Nardostachys jatamansi is a perennial herb found in western and northern China, Bhutan, India, and Nepal. The roots and rhizomes of *N. jatamansi* are utilised in traditional Chinese medicine for their calming and tonic properties. In Ayurvedic and Unani medicine, it is also used as a stimulant, antiseptic, insect repellent, and for the treatment of epilepsy, hysteria, convulsive disorders, stomachache, constipation, and cholera. Phenolic compounds, caffeoylquinic acid derivatives, lignans, neolignans, monoterpenoids, sesquiterpenoids, diterpenoids, and iridoids were discovered in previous phytochemical studies of *N. jatamansi* (Kumar *et al.,* 2018).

3. *HEMIDESMUS INDICUS* (NANARI)

According to the diverse biological activities attributed to its various parts, especially the roots, *Hemidesmus indicus* (L.) R. Br. ex Schult. (Apocynaceae) is widely employed in traditional medicine in several parts of the Indian subcontinent. Anti-arthritic, mosquitocidal, hepatoprotective, anti-cancer, anti-mutagenic, anti-acne, radioprotective, and anti-thrombotic properties have been found in plant extracts and formulations (Mahalingam *et al.,* 2008).

4. *ECLIPTA PROSTRATA* (KARISALANKANNI)

The Asteraceae family includes *Eclipta prostrata Roxb*. It is considered a weed of ethnomedicinal value. It is recognised in the Indian subcontinent's three major traditional medicinal systems: Ayurveda, Unani, and Siddha. Although the plant or plant parts are used to treat a variety of diseases, ethnomedicinal reports indicate that the most common uses are for gastrointestinal disorders, respiratory tract disorders (including asthma), fever, hair loss and greying, liver disorders (including jaundice), skin disorders, spleen enlargement, and cuts and wounds (Jahan *et al.,* 2014).

5. *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, tannins, saponins, proteins, carbohydrates and anthraquinone derivatives and, these are responsible for the pharmacological activity (Aparna *et al.,* 2018).

6. *BRASSICA ALBA* (WHITE MUSTARD)

The White mustard seeds are slightly larger than the other two kinds and are pale straw yellow in colour. Pungency is mild in white seeds. Mustard seeds are high in folate, niacin, thiamin, riboflavin, pyridoxine (vitamin B6), and pantothenic acid, which are all necessary vitamins. These vitamins are necessary in the sense that they must be replenished from outside sources. Vitamins in the B complex help in enzyme synthesis, nervous system function, and body metabolism regulation (Divakaran *et al.,* 2016).

7. *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 (Yadav *et al.,* 2016).

8. *LEUCAS ASPERA* (THUMBAI)

The plant *Leucas aspera* (Willd.) Linn. (Family: Lamiaceae), often known as 'Thumbai,' is found in India from the Himalayas to Ceylon. The herb has been used as an antipyretic and pesticide for centuries. Flowers are used as a stimulant, expectorant, aperient, diaphoretic, pesticide, and emmenagogue, among other things. Chronic rheumatism, psoriasis, and other chronic skin eruptions may benefit from the leaves. Snake bites are treated with bruised leaves (Prajapati *et al.,* 2010).

9. *SOLANUM TRILOBATUM* (THUTHUVALAI POWDER)

The plant *Solanum trilobatum* is commonly utilised in Indian traditional medicine. *Solanum trilobatum* is a member of the Solanaceae family, which is also known as Alarka. It is mostly used to treat respiratory illnesses such as bronchial asthma. The varied qualities of this plant have been documented in several papers. *S. trilobatum* has antioxidant properties, hepatoprotective properties, and protects mice from UV-induced damage and radiation-induced toxicity. Anti-inflammatory and antibacterial activities were discovered in *S. trilobatum* aqueous and solvent extracts (Ranjith *et al.,* 2010).

10. *TINOSPORA CORDIFOLIA* (SEENTHIKODI POWDER)

Tinospora cordifolia, also known as Seenthikodi, is a big, deciduous climbing shrub with greenish yellow characteristic flowers that grows at higher altitudes and belongs to the Menispermaceae family. Researchers from all over the world are intrigued by the plant's anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, antiallergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory, and anti-neoplastic qualities (Saha *et al.*, 2012).

2.5 ASSAYS

2.5.1. CAGE TEST

The mosquitoes utilised in the experiment must be pathogen- free. According to WHO guideline for efficacy repellants, 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* 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 *Cymbopogon citratus* computer incense bar was 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).

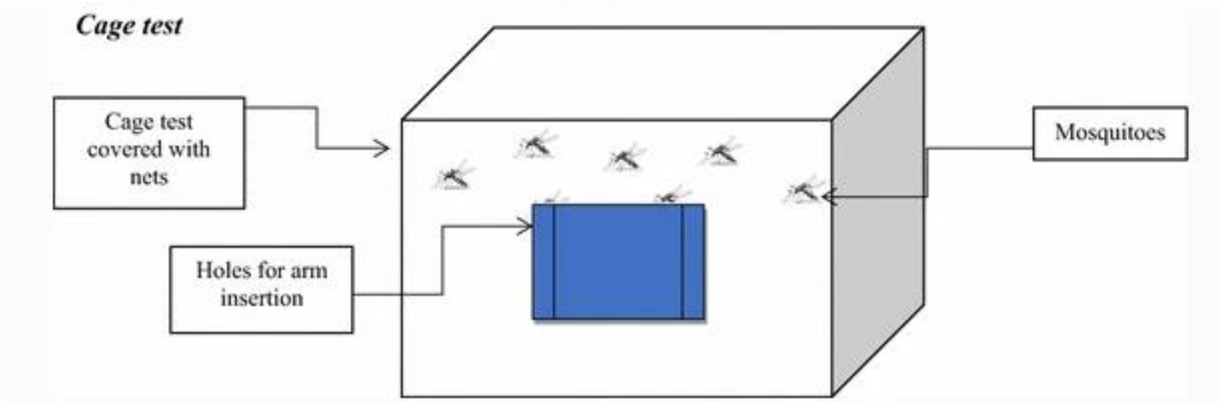


Figure: 7 – Cage for mosquitoes

2.5.2. 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 without incense bar to area with 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 h before the test. The behaviour of mosquito was observed in term of number of escaped mosquitoes to another space and remain 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 repellency was calculated using the formula (Adeela *et al*., 2016).

% Mosquito repellency: (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.

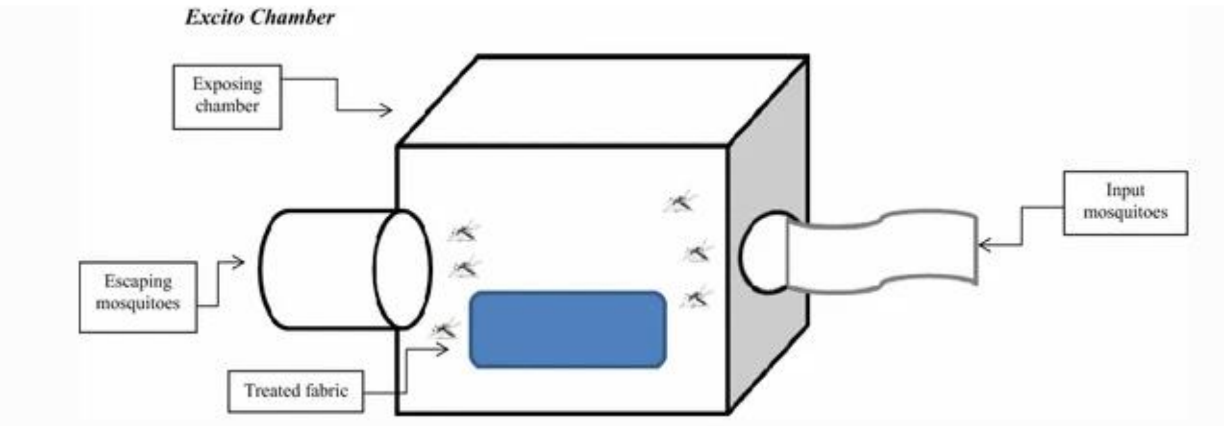


Figure: 8 – Excito – Chamber test

METHODOLOGY

3. METHODOLOGY

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

3.1 Plant collection

Fully completed fresh leaves of *Cymbopogon citratus* were collected from nearby Nursery Garden, Coimbatore. 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, *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°C which is similar to the environment from which they are isolated. The larvae were reared in 500ml white plastic container. Net is used to cover the container 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 to avoid fermentation and development of fungus. Larvae pupate within 3 – 4 days and pupae emerge 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 to transfer all the emerged adult mosquitoes into the cage (Umar *et al.,* 2021).

3.3 Larvicidal Activity

[A]. Preparation of Aqueous extracts:

The plant sample was collected from nearby garden in Coimbatore. 5g of fresh samples were collected, cleaned, grinded and extracted using distilled water. The extract was evaporated to dryness at 60°C 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 Coimbatore. 5g of fresh samples were collected, cleaned, grinded and extracted using ethanol. The extract was evaporated to dryness at 60°C and concentrations ranging from 20mg were dissolved in 5µl of dimethyl sulfoxide. Similarly, the plant extract was prepared by using ethyl acetate and chloroform

3.3.1. Biological assay

Two different concentration of extract was prepared in distilled water. All experimental exposures were made in petri - plate. Ten (10) larvae were collected with a Pasteur pipette and placed in a petri - plate containing different test sample and tested for larvicidal activity of *Aedes spp* and *Culex spp*. 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 *Cymbopogon citratus and* the percentage mortality was recorded (Ponkiya et al., 2018).

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

C = Number of larvae survived in the control

T = Number of larvae dead in the test sample

3.4. Preparation of incense bar

3.4.1. Preparation of mosquito incense bar paste

Herbal base preparation

Cymbopogon citratus leaves were collected from Nursery Garden, Coimbatore. They were cut into small pieces and shade dried for about two days. The dried parts were pounded and powdered using domestic grinder. Along with this, powdered forms of *Crysopogon zizanioides* (vetiver), *Indian catmin* (peimiratti), White mustard (venkadugu), *Erucastrum gallicum* (naaikadugu), seeds of *Lawsonia innermis* (henna), *Nardostachys jatamansi* (jadamanji), *Senna auriculta* (aavaram 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 C*edrus 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), *Cymbopogon citratus* (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.

Panchakavyam 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 in making *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 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 9 Computer Incense Bar

(a) Herbal base (b) Panchakavyam with herbal base (c) *Cymbopogon citratus* Panchakavyam & herbal base

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 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 *Cymbopogon citratus* computer incense bar was 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 without incense bar to area with incense bar. However, excite - 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 mosquito was starved overnight or least minimum 4 hour before the test. The behaviour of mosquito was observed in term of number of escaped mosquitoes to another space and remain 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 repellency was calculated using the formula (Adeela *et al.*, 2016).

% Mosquito repellency: (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 repellency and compared with incense bar made with *Cymbopogon citratus* 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 *Cymbopogon citratus* computer incense bar was 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 spread some of humanity's most serious infectious diseases, such as malaria, which kills between 0.6 and 1.2 million people each year, the majority of whom are children in low-income nations. It is becoming increasingly clear that no one action will be sufficient to eradicate malaria, and that a multifaceted approach, including vector control, is required. There are currently very efficient vector control strategies available, the most of them use insecticides, though there is evidence of resistance spreading. A number of innovative genetic techniques to vector control are now being tested. Massive investments in molecular resources, especially the distribution of several full-genome sequences, have tremendously aided mosquito research. Vector management is population biology in action, and I believe that continued success will necessitate paying as much attention to mosquito ecology as mosquito molecular biology (Charles *et al*., 2012).

Nature is an endless source of inspiration for both individual's 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 commercialized. 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 *A. gambiae*, implying that the kind of carbon where the hydroxyl substitution is present influences repellency (Khater *et al*., 2016)

The plant material of the genus *Cymbopogon* are of prolific occurrence and about 15 species of them have been chemically examined. The chemical constituents and their biological activity of *Cymbopogon citratus* 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 *Cymbopogon citratus,* which is known to have potential for pest control and insect repellency (Amancharla *et al.*, 2000)

The present study aimed to evaluate a larvicidal activity, mosquito repellent activity of incense bar made by using *Cymbopogon citratus leaves* and the result obtained are presented in this chapter.

4.1. Larvicidal activity

Two different concentration of extract was prepared in distilled water. All experimental exposures were made in petri- plate. Ten (10) larvae were collected with a Pasteur pipette and placed in a petri- plate containing various concentration of crude extracts. 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 *Cymbopogon citratus* and the percentage mortality was recorded (Ponkiya et al., 2018).

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

C = Number of larvae survived in the control

T = Number of larvae dead in the test sample

Table: 4.1.a - Larvicidal activity of Aqueous, Ethanol, Chloroform,Ethyl acetate extract of *Cymbopogon citratus* against *Aedes spp*

Extract	Concentration (μg/ml)	No. of larvae exposed	No. of larvae survived	Time duration	Percentage mortality
Distilled water (control)	-	10	0	24	0
Aqueous Extract	20 μg	10	8	24	20
	40μg	10	7	24	30
Ethanol	20μg	10	6	24	40
	40μg	10	5	24	50
Chloroform	20μg	10	8	24	20
	40μg	10	6	24	40
Ethyl acetate	20μg	10	8	24	20
	40μg	10	6	24	40

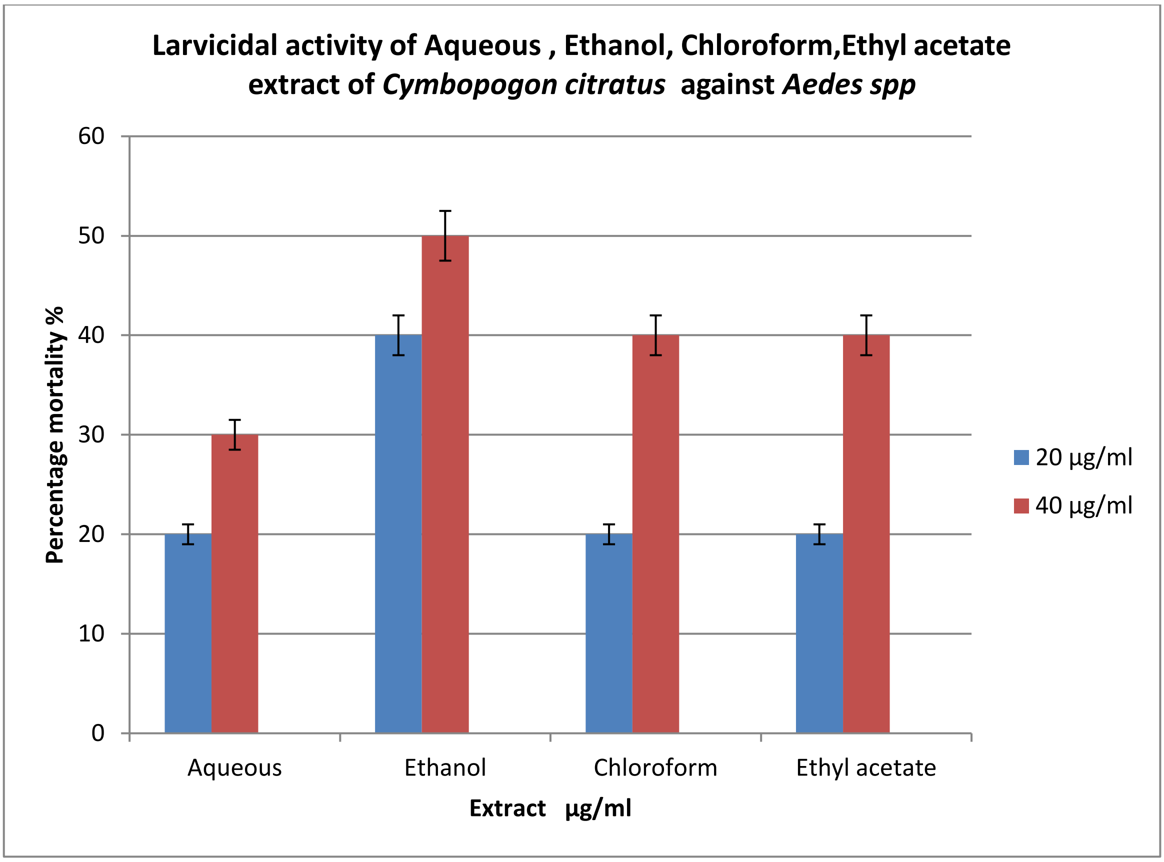
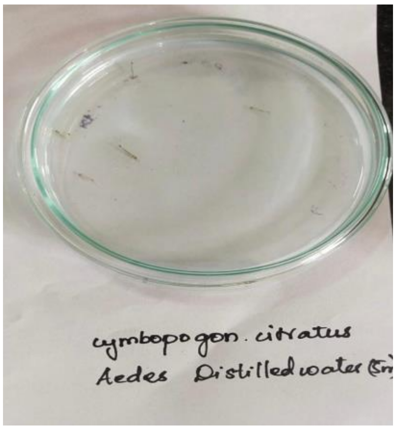


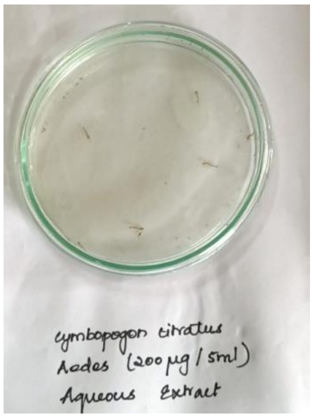
Figure 10 Graphical representation of Larvicidal activity of Aqueous, Ethanol, Chloroform,Ethyl acetate extract of *Cymbopogon citratus* against *Aedes spp*

Figure: 11 Larvicidal activity of aqueous, ethanol, chloroform, ethyl acetate extract of *Cymbopogon citratus* against *Aedes spp*

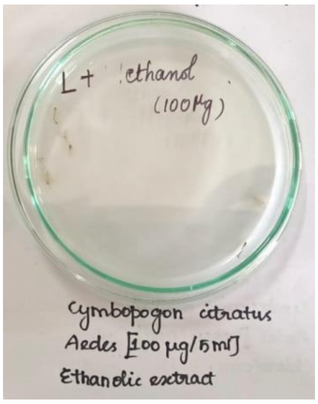


Distilled water

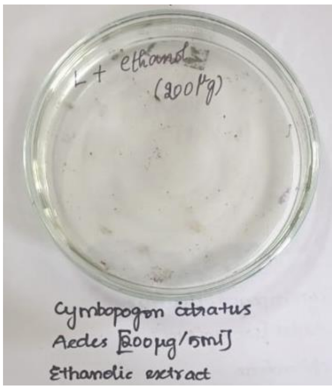
Aqueous extract 20µg



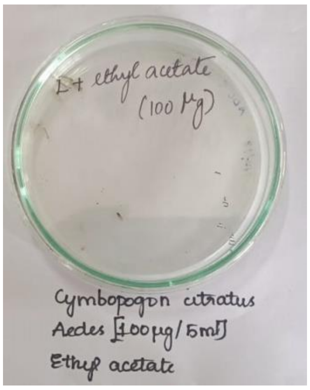
Aqueous extract 40µg



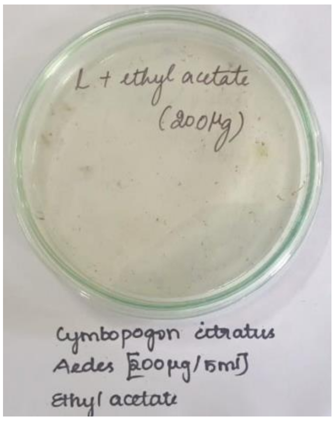
Ethanolic extract 20µg



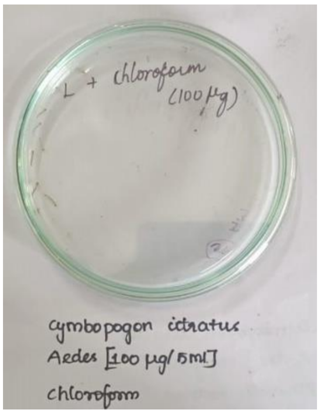
Ethanolic extract 40µg



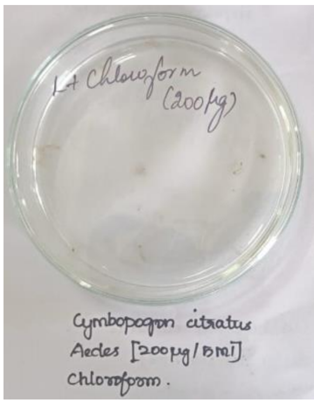
Ethyl acetate 20µg



Ethyl acetate 40µg



Chloroform extract 20µg



Chloroform extract 40µg

Table: 4.1.b - Larvicidal activity of Aqueous, Ethanol, Chloroform,ethyl acetate extract of *Cymbopogon citratus* against *Culex spp*

Extract	Concentration (µg/ml)	Number of larvae		Time duration	Percentage mortality
		Exposed	Survived		
Distilled water	-	10	0	24	0
Aqueous extract	20µg	10	8	24	20
	40µg	10	7	24	30
Ethanol	20µg	10	5	24	50
	40µg	10	4	24	60
Chloroform	20µg	10	6	24	40
	40µg	10	5	24	50
Ethyl acetate	20µg	10	7	24	30
	40µg	10	4	24	60

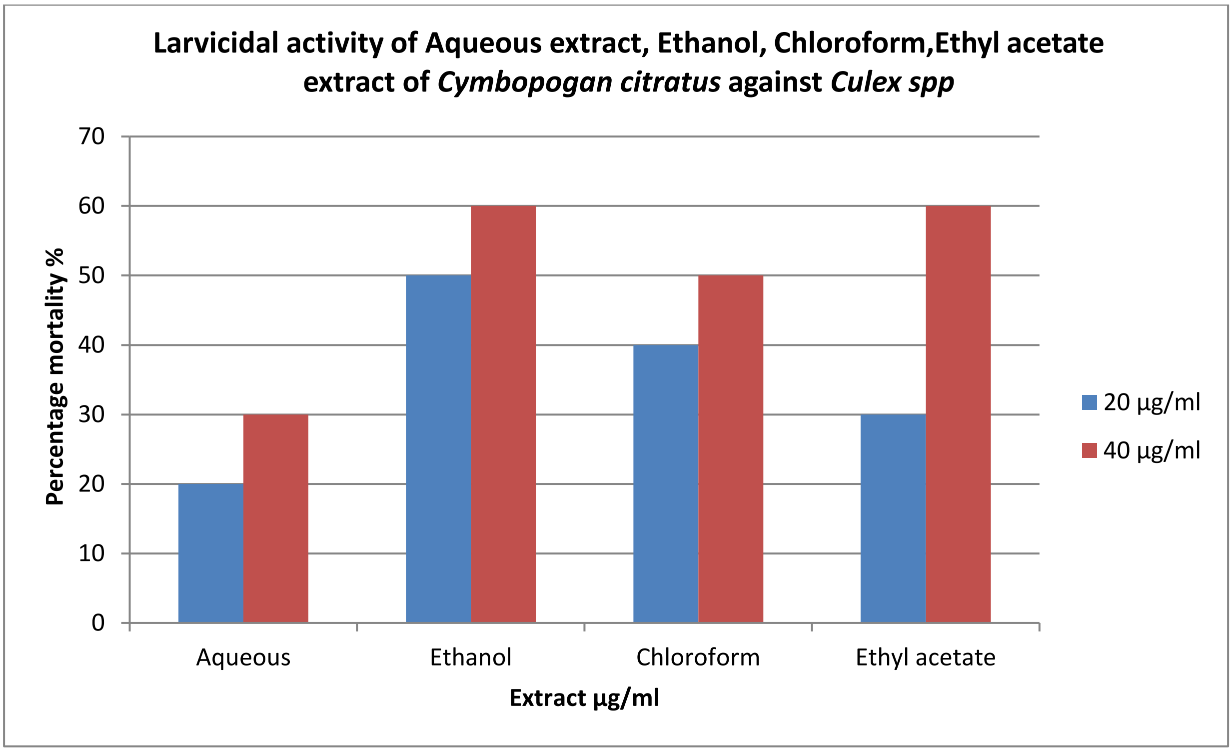
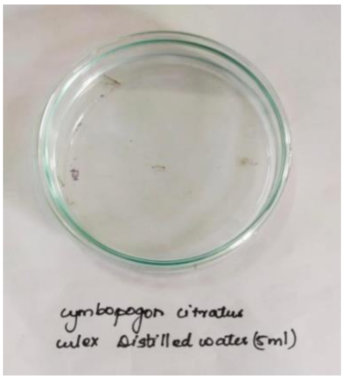
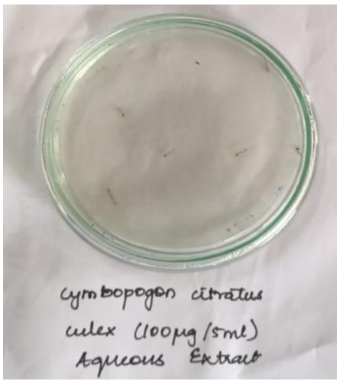


Figure 12 Larvicidal activity of Aqueous extract, Ethanol, Chloroform,Ethyl acetate extract of *Cymbopogan citratus* against *Culex spp*

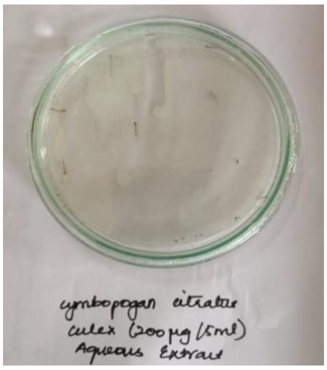
Figure: 13 Larvicidal activity of aqueous, ethanol, chloroform, ethyl acetate extract of *Cymbopogon citratus* against *Culex spp*



Distilled water



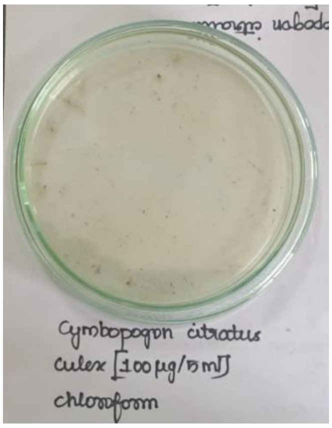
Aqueous extract 20µg



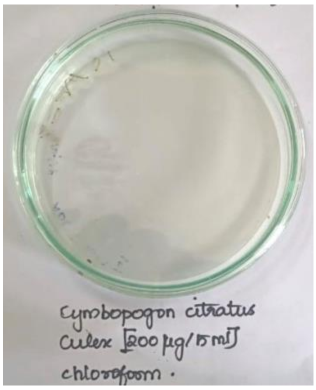
Aqueous extract 40µg

Ethanolic extract 20µg

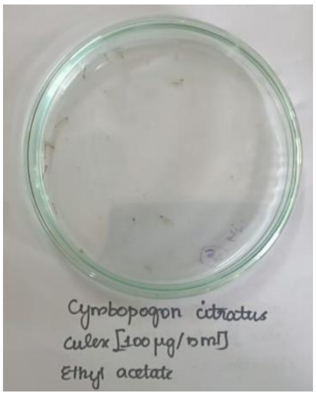
Ethanolic extract 40µg



Chloroform extract 20µg



Chloroform extract 40µg



Ethyl acetate 20µg

Ethyl acetate 40µg

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

With this observation we can say that *Cymbopogon citratus leaves* displays mosquito repellent activity and it can be used to produce commercially available incense bar. From these above readings, the larvicidal activity of Aedes was checked using the extract prepared in aqueous extract, ethonolic extract, chloroform and ethyl acetate at the rate of 20 µg/ml and 40 µg/ml. The ethyl acetate extract has shown 80% mortality mortality of larvae against the concentration of 20 µg/ml.

From these above readings, the larvicidal activity of culex was checked using the extract prepared in aqueous extract, methonolic extract, chloroform and ethyl acetate at the rate of 20 µg/ml and 40 µg/ml. The ethyl acetate extract has shown 80% mortality mortality of larvae against the concentration of 40 µg/ml.

4.2 Mosquito repellent activity

4.2.1 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 *Cymbopogon citratus* computer incense bar was 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).

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

Incense bar	No.of.Mosquito exposed	No of Mosquitos 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 *Cymbopogon citratus* leaves powder.	**20**	**4**	**7**	**7**

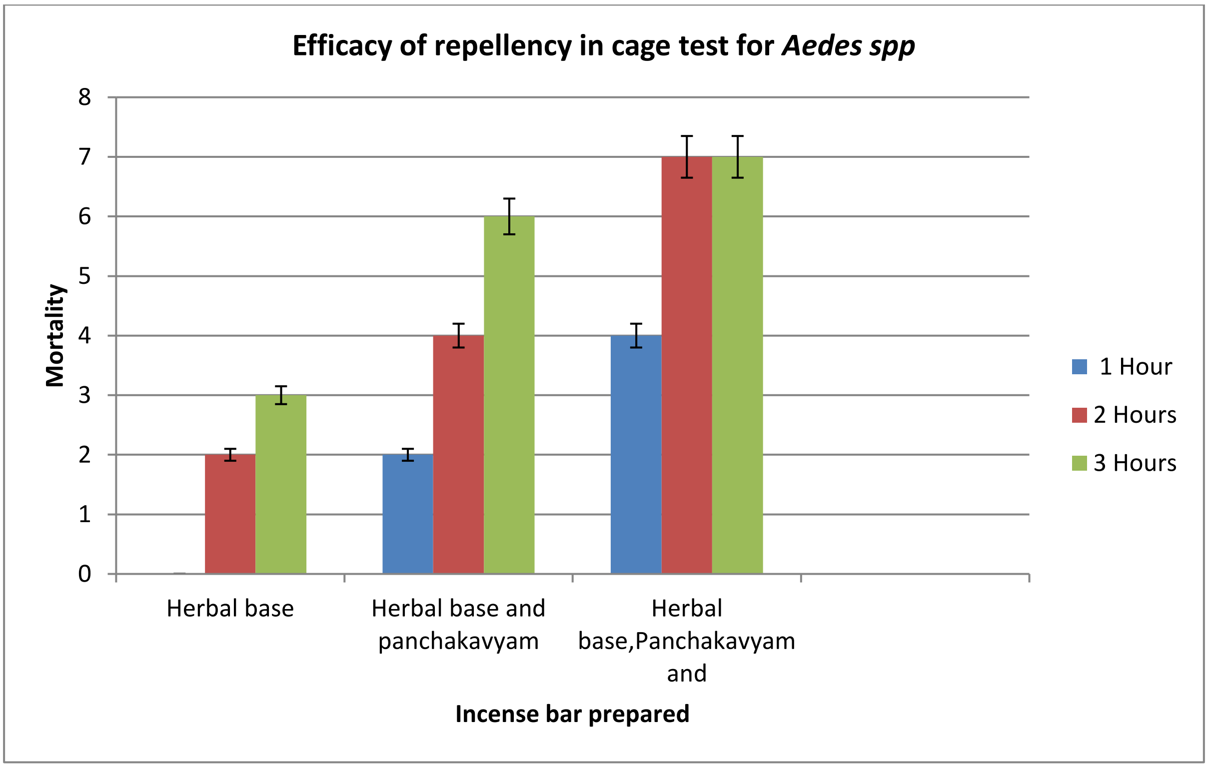


Figure 14 Efficacy of repellency in cage test for *Aedes spp*

Figure 15 Herbal Base

Figure 16 Incense bar using herbal base and panchakavyam

Figure 17 Incense bar using herbal products, panchakavyam and *Cymbopogon citratus* leaves

Table 4.2.2. Cage test for *Culex spp*

Incense bar	No.of Mosquito exposed	No.of Mosquitos died after		
		1 hour	2 hours	3 hours
Herbal base	**20**	**0**	**3**	**3**
Herbal base and panchakavyam.	**20**	**2**	**3**	**4**
Herbal base panchakavyam and *Cymbopogon citratus* leaves powder.	**20**	**4**	**6**	**8**

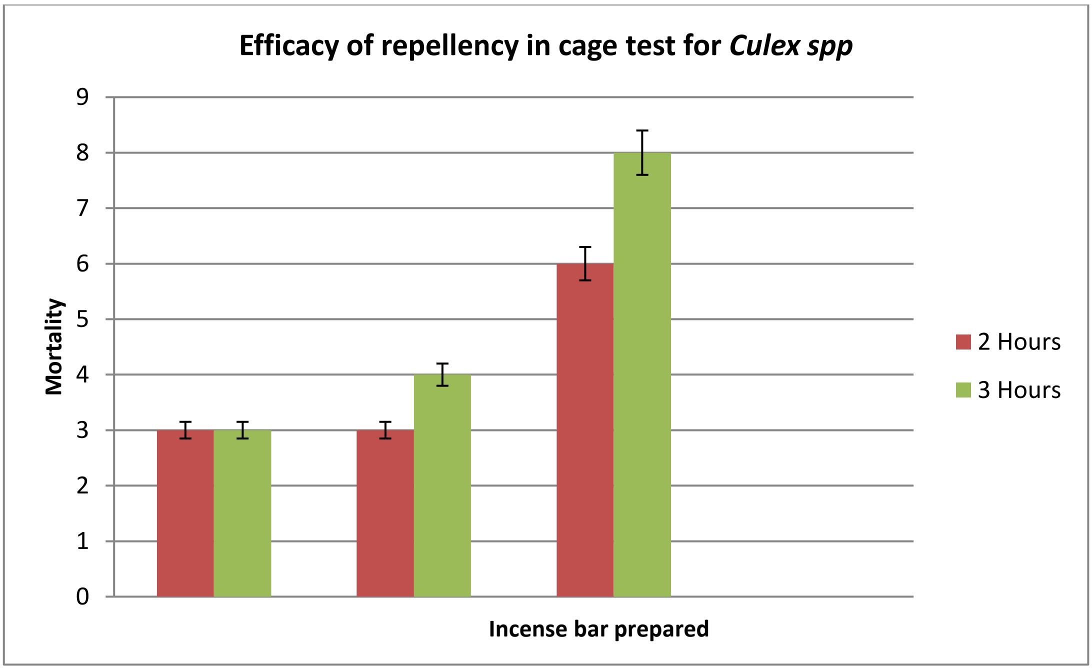


Figure 18 Efficacy of repellency in cage test for *Culex spp*

Figure 19 Incense bar using herbal base

Figure 20 Incense bar using herbal base and panchakavyam

Figure 21 Incense bar using herbal base, panchakavyam and *Cymbopogon citratus* leaves

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. (Ponkiya *et al*., 2018)

A new natural mosquito repellent was isolated from fresh leaves of *Cymbopogon 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* (Watanabe *et al*. 1995). The repellent efficacy of leaf extract of *Cymbopogon citratus* is also comparable with CO_2 extract of the seeds of the Mediterranean plant *Cymbopogon agnus- castus* (monks pepper; Mehlhorn *et al*. 2005). The above-cited studies undoubtedly indicated that Cymbopogon plants possess promising potent repelling activity against mosquitoes and other bloodsucking insect.

The present study indicates that incense bar using herbal products kill 4 *Aedes spp* mosquitoes in 3 hours, and the incense bar with herbal products and panchakavyam kill 10 *Aedes spp* mosquitoes in three hours. And the incense bar with herbal products, panchakavyam and *Cymbopogon citratus leaves* kill 19 *Aedes spp* mosquitoes in 3 hours. The incense bar using herbal products kill 6 *culex spp* mosquitoes in 3 hours, and the incense bar with herbal products and panchakavyam kill 8 *culex spp* mosquitoes in three hours. And the incense bar with herbal products, panchakavyam and *Cymbopogon citratus* leaves kill 19 *culex spp* mosquitoes in 3 hours. With this observation, we can say that *Cymbopogon citratus* 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. A very important point is that the experimental products have not shown any side effect on the users.

4.2.2 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 without incense bar to area with 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 mosquito was starved overnight or least minimum 4 h before the test. The behaviour of mosquito was observed in term of number of escaped mosquitoes to another space and remain 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 three times. The percentage of mosquito repellency was calculated using the formula. (Adeela et al., 2016).

% Mosquito repellency: (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.

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

Time (hours)	NEX	NES	NDE	Mosquito repellency %
1	20	3	2	25
2	20	4	5	45
3	20	6	6	60

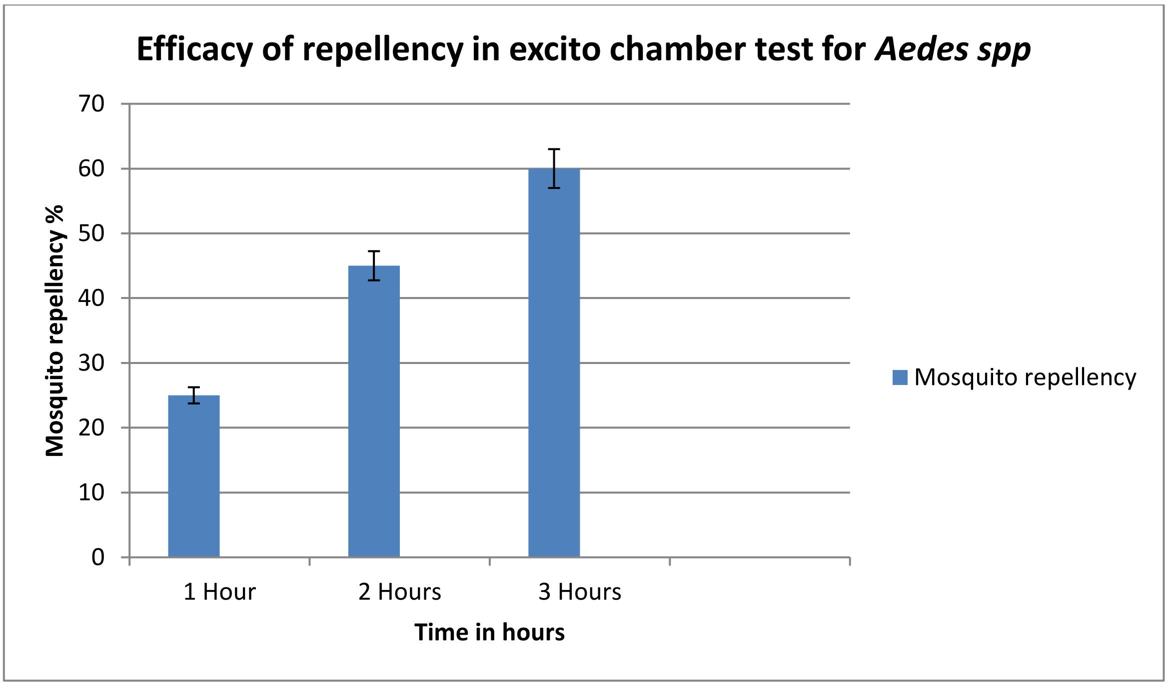


Figure 22 Efficacy of repellent in excito - chamber test for *Aedes spp*

Figure 23 Excito-chamber test for *Aedes spp*

4.2.4 Table - Excito - Chamber test for *Culex spp*:

Time (hours)	NEX	NES	NDE	Mosquito repellency %
1	20	3	2	25
2	20	5	3	40
3	20	7	4	55

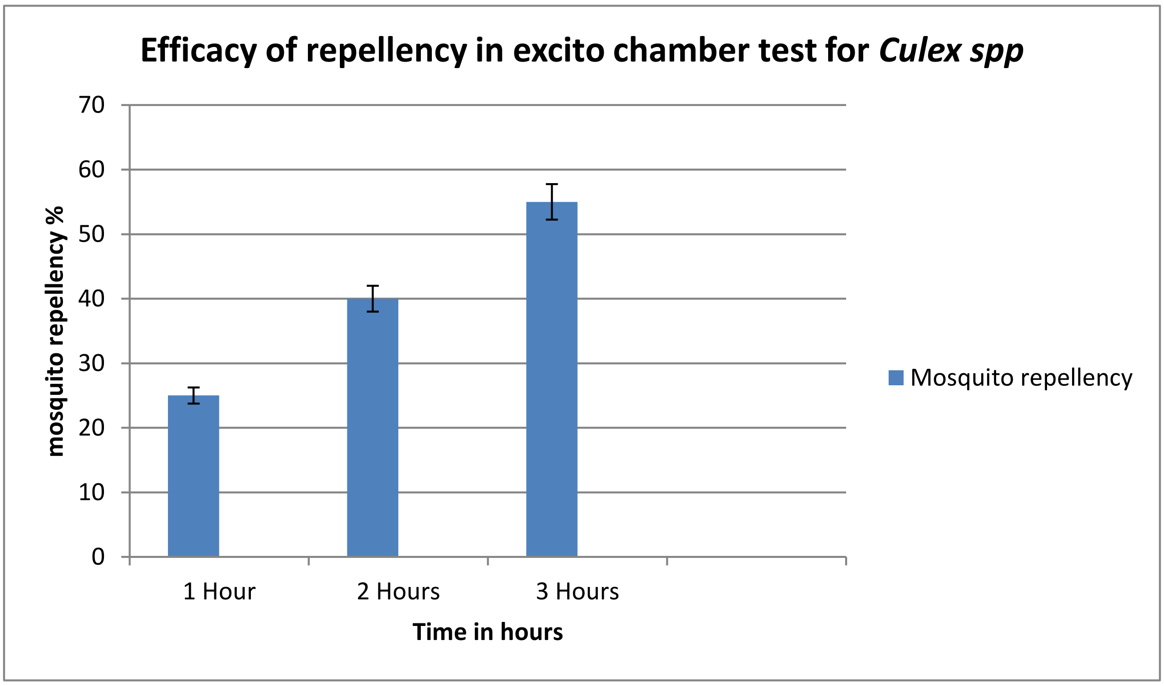


Figure 24 Excito - Chamber test for *Culex spp* mosquitoes

Figure 25 Excito – chamber test for *Culex spp*

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 (Ponkiya *et al*., 2018)

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 eco-friendly substances of leaves of *Cymbopogon citratus* for the control of vector mosquitoes. Plants can provide safer alternatives for modern deadly poisonous synthetic chemicals (Karunamoorthi *et al*., 2008)

The present study indicates that incense bar using herbal products, panchakavyam and *Cymbopogon citratus leaves* show 20% repellency in 1 hour, 30% repellency in 2 hours and 50% repellency in 3 hours against *Aedes spp* The incense bar using herbal products, panchakavyam and *Cymbopogon citratus leaves* show 30% repellency in 1 hour, 40% repellency in 2 hours and 45% repellency in 3 hours against *Culex spp*

4.2.3 Comparative studies

In this study we are comparing the commercially available mosquito repellent coil with the computer incense bar of *Cymbopogon citratus 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 *Cymbopogon citratus* computer incense bar was 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)

Table 4.2.5. Cage test for comparative studies:

Mosquito coil	No. of Mosquito exposed	No. of Mosquito dead
Goodnight	**25**	**25**
Maxo	**25**	**25**
***Cymbopogon citratus* Computer incense bar**	**25**	**20**

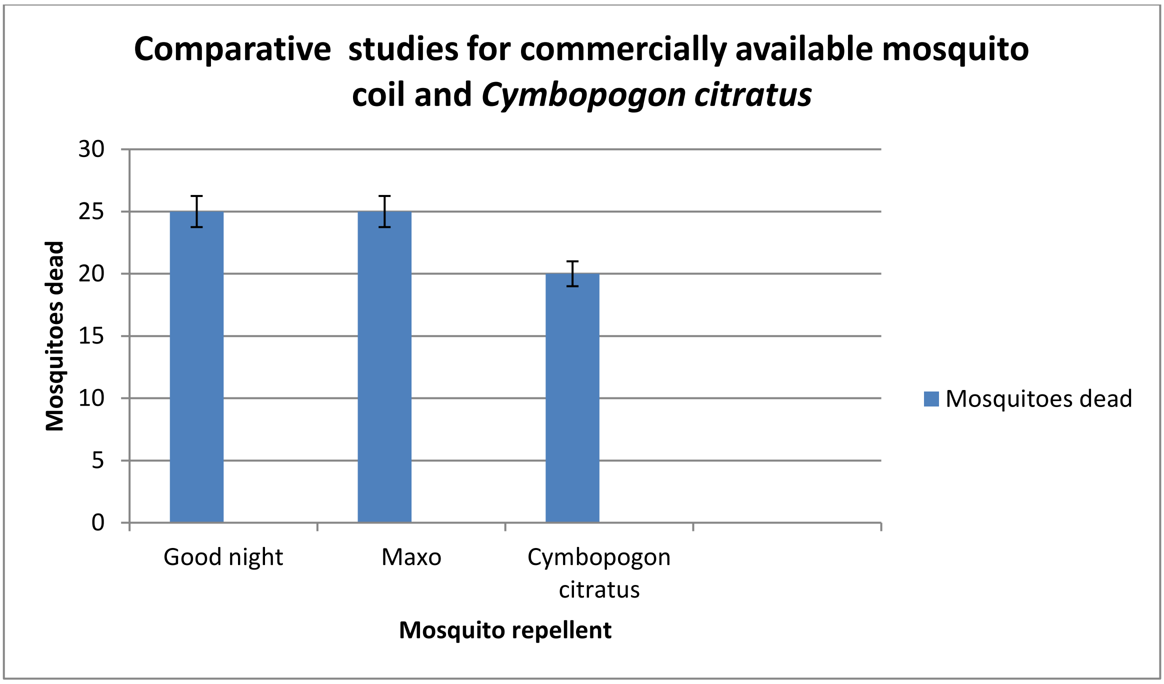


Figure 26 Comparative studies for commercially available mosquito coil and *Cymbopogon citratus*

Figure 27 Good night mosquito repellent activity

Figure 28 Maxo mosquito repellent activity

Figure 29 *Cymbopogon citratus* incense bar mosquito repellent activity

Herbal 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 (Karunamoorthi *et al*., 2016).

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 (Ponkiya et al., 2018).

This study reveals that computer incense bar equally kills the mosquitoes of the commercially available mosquito coil. In the present investigation, we have identified eco-friendly substances leaves of *Cymbopogon citratus* 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 *Cymbopogon citratus* 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 *Cymbopogon citratus*, which is known to have potential for pest control and insect repellency.

Local rural population are widely using leaves of *Cymbopogon citratus* to repel insects by mixing leaves with grain stores and fresh leaves are burnt with grass as a fumigant against mosquitoes. *Cymbopogon citratus* 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 *Cymbopogon citratus.* 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 *Cymbopogon citratus* 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 spp* 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 *Cymbopogon citratus* 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 *Cymbopogon citratus* kills 3, 4, 5 mosquitoes in 3 hours respectively. From this observation we can say that *Cymbopogon citratus* 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 using herbal products, panchakavyam and *Cymbopogon citratus* leaves show 20% repellency in 1 hour, 30% repellency in 2 hours and 50% repellency in 3 hours against *Aedes spp* The incense bar using herbal products, panchakavyam and *Cymbopogon citratus* leaves show 30% repellency in 1 hour, 40% repellency in 2 hours and 45% 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 *Cymbopogon citratus* 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.,

BIBLIOGRAPHY

BIBLIOGRAPHY

Amin Asadollahi, Mehdi Khoobdel, Alireza Zahraei-Ramazani, Sahar Azarmi and Sayed Hussain Mosawi (2019). Effectiveness of plant-based repellents against different Anopheles species: a systematic review. 18:436

Anuar, A.A., Yusof, N. Methods of imparting mosquito repellent agents and the assessing mosquito repellency on textile. Fash Text 3, 12 (2016).

Aurfa Adeela Anuar and Nuarain Yusof, (2016) 3:12.Methods of imparting mosquito repellent agents and the assessing mosquito repellency on textile, Fashion and textile, 14 :9-12.

Balaji APB , Polymeric nanoencapsulation of insect repellent : Evaluation of its bioefficacy on culex quinquefasciatus(2017) Journal of king saud university-science. 29: 517-527

Bamou R, Mayi M P A, Djiappi-Tchamen B, Nana-Ndjangwo S M, Nchoutpouen E, Cornel A J, Awono-Ambene P, Parola P, Tchuinkam T, Antonio-Nkondjio C (2021). An update on the mosquito fauna and mosquito-borne diseases distribution in Cameroon. Parasit Vectors. 14(1):527.

Braack L, de Almeida APG, Cornel AJ, Swanepoel R, de Jager C. Mosquito-borne arboviruses of African origin: review of key viruses and vectors. Parasite Vectors. 2018; 11:29.

Braack L, de Almeida APG, Cornel AJ, Swanepoel R, de Jager C. Mosquito-borne arboviruses of African origin: review of key viruses and vectors. Parasite Vectors. 2018; 11:29.

D.Raj Mohan and M.Ramaswamy,(2007). Evaluation of larvicidal activity of the leaf extract of weed plant, Ageratina Adenophora, against two important species of mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus, Academic journals,* 637:633-635

DeGennaro M. The mysterious multi-modal repellency of DEET. Fly (Austin). 2015;9(1):45-51.

Dhar R, Dawar H, Garg SS, Basir F, Talwar GP (1996). Effect of volatiles from neem and other natural products of gonotrophic cycle and oviposition of Anopheles stephensi and Anopheles culicifaciens. J. Med. Entomol. 33:257-263.

Dua VK, Nagpal BN, Sharma VP (1995). Repellent action of neem creem against mosquitoes. Indian J. Malariol. 32:47-55

Godfray H C (2013). Mosquito ecology and control of malaria. J Animal Ecology, 82(1):15-25.

Griffin M. Dill (2011). Insect Repellents, Pest Management Fact Sheet number 5108.

Hanem Fathy Khater, Abdelfattah M., Selim, Galal A. Abouelella, Nour A. Abouellela, Kadarkarai Murugan, Nelissa P. Vaz and Marimuthu Govindarajan (2017). Commercial Mosquito repellents and their safety concerns.

Henderson JP, Westwood R, Galloway T. An assessment of the effectiveness of the mosquito magnet pro model for suppression of nuisance mosquitoes. J Am Mosquito Control Assoc. 2006; 22:401–7

Islam J et al. Protection against mosquito vectors Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus using a novel insect repellent, ethyl anthranilate. Acta Tropica. 2017;174:56-63

Jitendra Kumar. Formulation of low smoke herbal mosquito repellent sticks by using different essential oils (2018). Research Gate, 7(4): 173-174

Klun JA et al. Comparative resistance of Anopheles albimanus and Aedes aegypti to N,N-diethyl-3-methylbenzamide (Deet) and 2-methylpiperidinyl-3-cyclohexen-1-carboxamide (AI3-37220) in laboratory human-volunteer repellent assays. Journal of Medical Entomology. 2004;41(3):418-422

Kumar S, Sharma A, Warikoo R (2018) Potential of Nardostachys jatamansi Extracts to Manage Indian Strain of Aedes aegypti: A Novel Approach for Vector Control. Vector Biol J 3:1.

Laha RC, De Mandal S, Ralte L, Ralte L, Kumar NS, Gurusubramanian G, Satishkumar R, Mugasimangalam R, Kuravadi NA. Correction to: Meta-barcoding in combination with palynological inference is a potent diagnostic marker for honey floral composition. AMB Express. 2017 Oct 11;7(1):189.

Leal WS. The enigmatic reception of DEET - the gold standard of insect repellents. Curr Opin Insect Sci. 2014 Dec 1;6:93-98.

Lupi E, Hatz C, Schlagenhauf P. The efficacy of repellents against Aedes, Anopheles, Culex and Ixodes spp.—A literature review. Travel Medicine and Infectious Disease. 2013;11(6):374-411

M.Divakaran, K.N.Babu (2016) Encyclopedia of Food and Health, Pages 9-19

Maia MF, Moore SJ. Plant-based insect repellents: A review of their efficacy, development and testing. Malaria Journal. 2011;10(1):S11

Miro Specos MM, Garcia JJ, Tornesello J, Marino P, Della Vecchia M, Defain Tesoriero MV.et al, Microencapsulated citronella oil for mosquito repellent finishing of cotton textiles. Trans R Soc of Trop Med Hyg. 2010; 104:653-658.

Mulatier M, Ahoua Alou LP, Chandre F, Pennetier C, Dormont L, Cohuet A. Effect of DEET-multiple exposures on behavior and life history traits in the malaria mosquito Anopheles gambiae (s.s.). Parasit Vectors. 2018 Jul 25;11(1):432

Norris EJ, Coats JR. Current and future repellent technologies: The potential of spatial repellents and their place in mosquito-borne disease control. International Journal of Environmental Research and Public Health. 2017;14(2):124

Paluch, Gretchen & Bartholomay, Lyric & Coats, Joel. (2010). Mosquito repellents: A review of chemical structure diversity and olfaction. Pest management science. 66. 925-35. 10.1002/ps.1974.

Pavela R, Benelli G. Ethnobotanical knowledge on botanical repellents employed in the African region against mosquito vectors—A review. Experimental Parasitology. 2016;167(Suppl. C):103-108

Pavela R, Maggi F, Iannarelli R, Benelli G. (2019). Plant extracts for developing mosquito larvicides: From laboratory to the field, with insights on the modes of action. Acta Trop, 193:236-271

Portilla-Pulido, J. S., Castillo-Morales, R. M., Barón-Rodríguez, M. A., Duque, J. E., & Mendez-Sanchez, S. C. (2020). Design of a Repellent Against Aedes aegypti (Diptera:

Culicidae) Using in silico Simulations With AaegOBP1 Protein. Journal of medical entomology, 57(2), 463–476.

Prajapati MS, Patel JB, Modi K, Shah MB. Leucas aspera: A review. Pharmacogn Rev. 2010;4(7):85-87.

Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K. Larvicidal activity of some Euphorbiaceae plant extracts against Aedes aegypti and Culex quinquefasciatus Diptera: Culicidae. Parasitol Res. 2014; 102:867-73.

Ranjith, M. S., Ranjitsingh, A. J., Shankar, S. G., Vijayalaksmi, G. S., Deepa, K., Babu, K., & Sidhu, H. S. (2010). “Solanum trilobatum in the management of atopy: Through inhibition of mast cell degranulation and moderation of release of interleukins.” Pharmacognosy research vol. 2,1 (2010): 10-4.

Rehman JU, Ali A, Khan IA (2014). Plant based products: use and development as repellents against mosquitoes: A review *Fitoterapia*; 95:65-74

Reiter P. Global warming and malaria: Knowing the horse before hitching the cart. Malaria Journal. 2008;7(1):S3

Robbins P J, Cherniack M G (1986). Review of the biodistribution and toxicity of the insect repellent N,N-diethyl-m-toluamide (DEET). J Toxicol Environ Health, 18(4):503-25.

Rojas-Sandoval J, 2016. Cymbopogon citratus (lemongrass). Invasive Species Compendium. Wallingford, UK: CABI.

Roland Bamou, Marie Paul Audrey Mayi, Borel Dijappi –Tchamen, Stella Mariette Nana-Ndjangwo. An uptdate on the mosquito fauna and mosquito-borne disease distribution in camoroon (2021). Parasites and vetor 14:527

Rownak Jahan, Abdullah Al-Nahain, Snehali Majumder, and Mohammed Rahmatullah (2014). Review of Ethnopharmacological Significance of Eclipta alba (L.) Hassk. (Asteraceae), Article ID 385969

Saha S, Ghosh S. Tinospora cordifolia: One plant, many roles. Anc Sci Life. 2012 Apr;31(4):151-9.

Samapika Nandy, Anuradha Mukherjee, Devendra Kumar Pandey, Puj Ray, Abhijit Dey (2020). Indian Sarsaparilla (Hemidesmus indicus): Recent progress in research on ethnobotany, phytochemistry and pharmacology. Volume 254

Singh RK, Dhiman RC, Mittal PK (2006). Mosquito larvicidal properties of Momordica charantia Linn. (Family : Cucurbitaceae), J.Vect .Borne.Dis. 43:88-91.

Singh SP, Raghavendra K,Singh RK, Subbarao SK (2002).Studies on the larvicidal properties of Solanum nigrum Linn. (Family: Solanaceae). Curr.sci. 81:1529-1536.

Sohn, E. Malaria control: The great mosquito hunt. Nature 511, 144–146 (2014).

Umar, Ammar & Dankaka, Amina & Shah, Mohammad & Umar, A & Lamba, M. (2021). Journal of Global Agriculture and Ecology MOSQUITO REPELLENT ACTIVITY OF THE LEAF EXTRACTS OF *Cymbopogon citratus* AND *Eucalyptus globulus* against *Anopheles spp*.