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Toxicity and Biological Activities of *Artemisia arborescens* Leaf Extract Against Filarial Vector, *Culex quinquefasciatus* (Diptera:Culicidae)

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Abstract:

The present study evaluated the mosquitocidal, developmental, behavioral, and reproductive effects of ethanol leaf extracts of *Artemisia arborescens* against *Culex quinquefasciatus*. Larval bioassays revealed a strong dose-dependent increase in mortality, with the highest concentration (2%) producing 100%, 96%, 91%, and 85% mortality in the 1st to 4th instars, respectively, while controls exhibited no mortality. Pupae and emerging adults were similarly affected, where 2% extract caused 86% pupal mortality, 79% adult mortality, and reduced adult emergence to 52%, confirming marked toxicity across developmental stages. Behavioral assays showed significant adult repellency (76%) and oviposition deterrency (81%) at 2% extract, indicating strong interference with host-seeking and reproductive site selection. Developmental duration was progressively prolonged with increasing concentrations, with 2% extract extending instar durations to 7.3–10.2 days compared to 1.6–4.3 days in controls. Pupal and adult durations were similarly delayed, with total pupal duration reaching 10.6 days at the highest concentration. Reproductive parameters were notably suppressed, fecundity decreased from 205 eggs in controls to 86 eggs at 2% extract, while hatchability dropped sharply to 28%. Larval-pupal intermediates increased markedly in treated groups, reaching 75% at 2%, indicating strong developmental disruption. Additionally, biting deterrence increased in a concentration-dependent manner, peaking at 76% at 2%, highlighting the extract's potential as an adult behavioral deterrent. Overall, *Artemisia arborescens* ethanol leaf extract demonstrated significant larvicidal, pupicidal, developmental, fecundity-reducing, oviposition-deterring, and biting-preventive effects on *Culex quinquefasciatus*. These results indicate that the plant extract is a promising eco-friendly botanical candidate for integrated mosquito management programs.

Key words
Artemisia arborescens, *Culex quinquefasciatus*, larvicidal, pupicidal, adulticidal, reproductive activity, repellent.

1. INTRODUCTION

Mosquitoes are the important blood sucking arthropods that can transmit serious communicable diseases with many socioeconomic consequences. They are belonging to genera *Culex*, *Anopheles* and *Aedes* are acting as vectors for many diseases like, Malaria, Filariasis, Japanese Encephalitis, Dengue fever, Yellow fever etc. When compared to the arthropods, mosquitoes affect millions of people throughout the world by transmitting various communicable diseases. Thus, WHO has declared that the mosquitoes are the number one public enemy (WHO,1996). Over the 70 million people in 100 countries around the world were affected by vector borne diseases every year and 10.4 million of the Indian population. Mosquito borne diseases are spread globally, causing huge number of mortality and thereby acting as factors impedimenting the economic development of most of the developing countries across the world (Borah *et al*., 2012). Mosquitoes continue to pose a serious infectious threat to modern society despite efforts to eradicate them, harming not only people but also other animal species. These insects are ideal disease vectors because of their extraordinary capacity for environmental adaptation, rapid and widespread reproduction, tolerance to some pesticides, and modification of eating patterns to withstand control measures (Saleem and Lobanova, 2020).

Culex quinquefasciatus (Diptera: Culicidae) is the most wide spread mosquito in tropical and subtropical regions and breeds in wide range of stagnant water bodies such as cemented drains, ditches, pools, rice fields, river margins, marshes, wells and ponds (Andreadis, 2012; Ilahi and Suleman, 2013). Its irritating biting creates nuisance and is responsible for transmitting the pathogen of lymphatic filariasis (elephantiasis, *Wuchereria bancrofti*) (Ramaiah *et al*., 2003). Millions of people suffer from filariasis in different regions of the world (Simonsen, 2009). Few cases of tropical pulmonary eosinophilia in Pakistan have also been reported (Beg *et al*., 2001). *Cx. quinquefasciatus* is also known for transmitting malaria in birds (Glad and Crampton, 2015). The 90 million people worldwide were once infected with *Wuchereria bancrofti.* In India alone is 25 million people microfilaria and 19 million people suffer from filarial diseases (Ravichandran and Kanayairam, 2014). Respiratory trumpets of *Culex* mosquito are long and narrow. Adult *Cx. quinquefasciatus* vary from 3.96 to 4.25 mm in length. Kingdom: Animalia; Phylum: Arthropoda; Class: Insect; Order: Diptera*;* Family: Culicidae; Genus:*Culex* Species: *quinquefasciatus.*

Lately, there has emerged a pressing requirement to employ eco-friendly botanical solutions for controlling vector mosquitoes. This involves utilizing natural products derived from plants through physical and chemical means as a biological control approach. The use of natural products is necessary in order to prevent further hazardous effects on humans and resources. Moreover, the cost-effectiveness of utilizing, producing, and applying plants as pesticidal agents is notably lower in comparison to the detrimental impacts of chemicals, pollution, and radiation (Aljameeli, 2023). Insecticides derived from plants hold great potential as viable substitutes to synthetic mosquito control agents, thanks to their limited toxicity towards mammals and swift degradation (Njoroge and Berenbaum, 2019). Particularly, Eos have gained considerable attention as potential bioinsecticides.

Over time, synthetic insecticides like organophosphates, pyrethroids, and insect growth regulators have been employed to eliminate both the larvae and adult of *Ae. aegypti* mosquitoes (Algamdi and Mahyoub, 2022). Through the utilization of these insecticides, it is possible to achieve effective pest management. Nevertheless, the frequent application of these chemicals has posed a threat to the balance of biological ecosystems, leading to the extensive emergence of resistance and resurgence of mosquito-borne diseases (Sarma *et al*., 2019). Insecticides also have harmful effects on non-target populations as they are non-selective. This gives rise to adverse outcomes, such as harm to non-target organisms, as well as heightened worries regarding environmental and human well-being. The financial implication of chemical insecticides application is burdensome to both public health organisations engaged in mosquito control and individual homeowners (Njoroge and Berenbaum, 2019). This has necessitated the exploration for eco-friendly, simpler, safer, sustainable alternative means for mosquito control.

Larviciding and IGRs are the options of first choice because the immature stages of mosquitoes are easily managed and controlled (WHO, 2005). The larvae breed in a series of water-holding containers which included disposed plastic basins, car tyres, plastic containers, buckets, earthenware pots, plastic barrels, metal drums, jerry cans and poly tank (Ojukwu *et al*., 2022). Mosquito bites may also cause allergic responses including local skin reactions and systemic reactions such as urticaria and angioedema (Peng *et al*., 2004). Botanical and microbial insecticides have been increasingly used for mosquito control because of their efficacy and documented nontoxic effects on nontarget organisms (Ascher *et al*., 1995). The highest number of malaria, *P. falciparum* cases and malaria-related deaths are recorded from the state of Orissa located in the eastern part of India (Sharma *et al*., 2009).

The effectiveness of several medicinal plant preparations as larvicidal agents or as mosquito repellents has not yet been determined. Recently, plant-based insecticides and those that don't hurt the environment have received increased attention while creating new pesticides (Pavela, 2015; Suman *et al*., 2012). Therefore, natural substances are the ideal alternative since they are eco-friendly, bio-sourced, and safe to use, causing less harm to the ecosystem and non-target species. Furthermore, a variety of extracts and compounds derived from diverse plant families have been investigated as possible new larvicides (Baz *et al*., 2024). Studies have revealed that saponin (Wiesman and Chapagain, 2006) isoflavonoids (Joseph *et al*., 2004), essential oils (Cavalcanti *et al*., 2004), alkaloids and tannins (Xing *et al*., 2004) are all effective against mosquito larvae.

Plant derived products have rich pools of chemical constituents that engage in diverse biological activities. These are considered safer than chemical products because of easy and quick degradability and comparatively lesser hazardous effects. The insecticidal, repellent and growth regulatory activity of various plant derivatives and phytochemical constituents have been reported against mosquitoes (Sukumar *et al*., 1991; Shaalan *et al*., 2005; Sharma *et al*., 2009). Since plant extracts contain a pool of constituents, identifying the bioactive constituents in these botanicals becomes significant. As the plant species, its geographical location, the part and solvent used for extraction, and the methodology adopted can affect its efficacy significantly, the foremost requirement is the preliminary screening of different plants' crude extracts and assessing their relative impact (Anibogwu *et al*., 2021). However, most such studies have been conducted with widely known economically and medically important flora. Since the commercial use of this vegetation can threaten these species and disturb the ecosystem, using weeds for mosquito management could be advantageous (Arunthirumeni *et al*., 2023).

Insecticides derived from medicinal plants are called botanical insecticides and they have been screened for their mosquitocidal activities (Bekele and Petros, 2017). Mahanta *et al*. (2019) reported biological effects caused by *Lippia alba* essential oils at various stages of *Cx. quinquefasciatus* and *Aedes aegypti.* Pratti *et al.* (2015) reported the larvicidal activity caused by *Schinus terebinthifolia* essential oils against *Ae. aegypti.* Fifty-six substances were identified which constituted the 81.67% of the essential oil composition. Bekele and Petros (2017) reported repellent activity on *Anopheles arabiensis* with *Brassica nigra;* and *Aloe pirottae* extracts. Jayapriya and Shoba (2015) studied various mosquitocidal activities of different solvent extracts of *Rhinacanthus nasutus* leaves against *Cx. quinquefasciatus* and *Ae. aegypti.*

The mosquito life cycle is a complex sequence of developmental stages, each demanding precise energy resource orchestration as developing mosquitoes experience aquatic and terrestrial factors. For example, Van Meer and colleagues demonstrated that fatty acid oxidation is important to generate energy for flight and reproduction (Van Meer *et al*., 2008). The maternal investment of lipids along with protein vitellogenin, set the stage for embryonic development. As new larvae emerge, they acquire lipids from the mother via egg lipid deposition (Atella and Shahabuddin, 2002). The lipid repertoire continues to play a pivotal role in sustaining energy demands during growth, moulting, and metamorphosis. For example, structurally, insect egg cuticles consist of a protective layer of chitin, covered in a waxed single-layered epithelium of fatty acids and sterols (Wrońska *et al*., 2023). As larvae progress through the four developmental stages in an aquatic environment, the ecdysteroid hormone crustecdysone (20-hydroxyecdysone, 20E) serves as an essential signal in maturation (Ekoka *et al*., 2021).

The chemical mode of controlling measures invokes undesirable effects in target and non-target organisms and also causes ill effects on the environment. Thus, there is an impetus on developing better controlling agents from natural sources specifically from plants. Several botanical pesticides exhibited deleterious effects on the growth and development of insects, by reducing the survival rates of larvae and pupae as well as lengthening the developmental stages and inhibited the adult emergence (Koul *et al*., 2008). The larval instars of *Cx. quinquefasciatus* exposed to alkaloid isolated from *Ageratum conyzoides* showed growth regulating and chemosterilant activities such as shortening of total developmental periods, reduced fecundity and fertility in female mosquitoes (Arya and Sahai, 2014).

According to Dua *et al*. (1996) the essential oil obtained from the leaves of *Aloysia citrodora* and *Artemesia arborescens* showed adulticidal activity against vectors of malaria *(An. Culicifacies, An. Stephensi),* vector of dengue and chikungunya (*Ae. Aegypti*) and vector of filariasis *(Culex quinquefasciatus)*. At a concentration of 400 ppm, the methanolic extract of *L. camara* leaves gave the highest protection against the bite of female *Anopheles* for more than five hours. Further, the flower extract of *L. camara* provides 94.5% protection against *Ae. aegypti* and *Ae. albopictus* (Dua *et al*., 1996). A study performed by (Oparaocha *et al*., 2010) showed that lotion prepared from the volatile oil of *O. gratissimum* provides effective protection against the bite of different mosquito vectors.

At very low concentrations these extracts inhibit emergence of treated larvae as disease transmitting adults see also (Pushpalatha and Muthukrishnan, 1999). Bioactive compounds in the neem kernel extracts have been reported to induce male sterility, oviposition repellency and suppress fecundity in *Culex tarsalis* and *Cx. quảnquefasciatus* (Su and Mulla,1998). Kumudini *et al*. (1994) found that treatment of *C. quinquefasciatus* adults with resespine significantly reduced fecundity. Aristolochic acid from *Aristolochia bracteata* was reported to induce sterility in *Aedes aegypti* (Bhaskar *et al*., 1979).

Mosquitoes are affected by phytochemicals in a number of ways, including as oviposition deterrent, developmental toxin, hatching blocker, adulticidal, ovicidal, and emergence blocker (Bakkali *et al*., 2008). Prajapati *et al.* (2005) reported that botanical insecticides also have the ability to deter the mosquitoes from egg laying. Active ingredients contained in plant extracts induced physiological and behavioral changes in the gravid female mosquitoes. The active ingredients of plant-based insecticides act as chemosterilent, growth regulators or they may exert olfactory stimuli acting as repellent (Prathibha *et al*., 2014). Plants that repel insects, also deter oviposition and inhibit adult emergence (Rajkumar and Jebanesan, 2009; Prathibha *et al*., 2014).

Vector control strategies focus on reduction of the population by inducing reproductive toxicity thereby decrease the fecundity and fertility rate of the insect species. Sterile Insect Technique is a good approach to attain this target because it is species specific, environmentally sound and effective tool to control mosquito population (Bouyer *et al*., 2020). Sterilization can be induced by irradiation or chemosterilization methods, which can induce toxicity on the reproductive cells of the insects (Bouyer *et al*., 2020). Li *et al*. (2023) suggest an innovative method which involve development of a molecular genetic control system termed precision guided sterile insect technic (pgSIT) in *Aedes aegypti.* However, the effort of implication of chemosterilants in field population among insects being largely abandoned due to some external factors as well as concerns regarding toxicity (Baxter, 2017).

Many proteases have emerged as prominent reproductive players in insects and mammals (Braswell *et al*., 2006; Dacheux *et al*., 2003). *Leucine aminopeptidases* (LAPs), which are members of the metalloprotease family, are capable of cleaving leucine residues located at the N-terminal end of peptides or proteins. In addition to their key role in peptide turnover, LAPs also have various functions in bacteria, mammals, and plants (Kloetzel and Ossendorp, 2004; Matsui *et al*., 2006). Notably, the neo- functionalization of LAPs have been demonstrated in sperm. In mammals, LAP activity in seminal plasma correlated with male fertility (Gonzalez Buitrago *et al*., 1985). Furthermore, proteomic analysis revealed a high abundance of LAP orthologs of sperm in Lepidoptera, Drosophila, and diverse mamma lian taxa (human, rat, mouse, and *Macaca mulatta*) (Baker *et al*., 2008; Whittington *et al.*, 2015; Zhao *et al*., 2017).

A single mating enables a female mosquito to store enough sperm in the spermathecae to contribute to fertilizing all the eggs in the female lifetime. Males transfer a mixture of seminal fluid and sperm directly into female spermathecae through insemination; the sperm then moves along the spermathecal ducts and are then released to fertilize eggs during ovulation (Durant and Donini, 2020). Structurally, there are three functional spermathecae in female *Ae. aegypti*: one large and two small (Pascini *et al*., 2012). Functionally, the secretions of spermathecal glands-containing energy metabolism enzymes, glycoproteins, and lipoproteins are responsible for sustaining and nourishing sperm that are stored in spermathecae (Heifetz and Rivlin, 2010; Baer *et al*., 2009). Furthermore, spermathecae protect sperm from oxidative stress and damage contributing to sperm viability and long term storage (Heifetz and Rivlin, 2010). Previous studies have shown that female fecundity is specifically reduced by double-stranded RNA (dsRNA) knockdown of glucose dehydrogenase (Gld), N-acetylgalactosaminyl transferase 6 (GALNT6), or Kazal-type serine protease inhibitor (KSPI), which were selected by RNA-seq analysis between virgin and inseminated female spermathecae (Pascini *et al*., 2020). Therefore, proteins related to fertility and fecundity in spermathecae may be potentially utilized as CRISPR/Cas9-targeted genes to control mosquito populations.

Insect repellents of plant origin have been used to protect humans from the bites of host-seeking mosquitoes (Maia and Moore, 2011). Such repellents may have a fundamental role in areas where malaria vectors are active during the early hours of the night, as the inhabitants are often outdoors at these times. Thus, they may serve as a complementary method to the indoor-based vector control method (Karunamoorthi *et al*., 2009). The use of traditional repellents to repel/kill mosquitoes and other blood-sucking insects is common in rural communities in Africa (Seyoum *et al*., 2002). For example, in Kenya, direct burning and placing branches or whole plants are the common methods to drive away mosquitoes (Seyoum *et al*., 2002). In Eritrea, hanging the different plant parts on the walls at the head and foot of beds reduces endophagic mosquitoes (Waka *et al*., 2004). In Tanzania, people burn or place fresh plant materials at selected places or spray to decrease the number of mosquitoes indoors at night (Innocent *et al*., 2014). In different parts of Ethiopia, smoking by burning various parts of repellent plants is the principal way of deterring nuisances and biting insects (Karunamoorthi *et al*., 2009; Innocent *et al*., 2014).

Essential oils (Eos) are volatile, aromatic liquids produced from plant material by steam distillation (El-Shourbagy *et al*., 2023). They are composed of a mixture of highly volatile and lipophilic components including sesquiterpenes, phenols, coumarins, monoterpenes, anthraquinones, and alkaloids (Rios, 2016; Sharifi-Rad *et al*., 2017). Many factors affect the chemical composition of Eos such as plant species and subspecies, part of the plant used, harvest time, geographical location, and the extraction methods used (Andrade-Ochoa *et al*., 2018). Eos are widely used in diverse commercial industries for numerous applications (e.g.. perfumes and cosmetics) and, due to their anti- oxidant and antimicrobial properties, are frequently sought for medicinal and pharmaceutical applications (Rios, 2016). In addition, they also have applications as insect repellents and/or insecticides that can disrupt insect behavior, physiology, and biochemistry as well as induce neurotoxic effects (Krzyżowski *et al*., 2020). The Eos have been shown to have adulticide, larvicide, deterrence, and repellence activities against mosquitoes (Andrade-Ochoa *et al*., 2018; de Souza *et al*., 2019). Furthermore, Eos are effective, renewable, biodegradable, non-persistent in the environment, and relatively. Safe for non-target organisms and humans (Jalali Sendi and Ebadollahi, 2014).

Therefore in the present study we have screened *Artemisia arborescens* leaves extract on the larvicidal, pupicidal, adulticidal, larval, pupal and adult duration, reproductive activity, repellency, biting deterrency and biochemical constituents of *Culex quinquefasciatus.* The potential result of the present study would be useful in promoting research and analysis aiming at the development of new agent for mosquito control support based on bioactive compounds from indigenous endemic medicinal plant source.

2. MATERIALS AND METHODS

2.1Collection and preparation of plant extracts

Healthy leaves of *Artemisia arborescens* were collected from Nilgiri hills of Tamilnadu, India. The plants were identified with the help of experts in the Department of Botany, Government Arts College, Udhagamandalam and standard books. The collected plant materials were washed in tap water, cut into small pieces and air dried. After the plants were completely dry, they have been ground into powder and then macerated in solvents (ethanol) at room temperature for 3 days and filtered. The combined filtrate were concentrated to dryness by rotary evaporation at 50ºC and kept in a freezer. In preparing test concentrations, plant extract was volumetrically diluted in ethanol.

2.2 Mosquito culture

Mosquito larvae/eggs of *Culex quinquefasciatus* have been collected in an around Ooty. The mosquito colonies were maintained at 27 $\pm$ 2 ^{0}C, 75-85% relative humidity index a 14:10 light/dark photo period cycle (Murugan and Jeyabalan, 1999).

2.3 Larvicidal and Pupicidal assays

Larvae tested for the present study was obtained from our laboratory culture. Freshly hatched or moulted larvae were used for the bioassay tests. The required quantity of plant extract concentrations were mixed thoroughly with 200 ml of rearing water in 500ml plastic troughs. One hundred early fourth instars mosquito larvae were released into each trough. Larva food consisted of 1g of finely ground dog biscuits per day per trough. Dried coconut midribs were place over water as the substratum for pupation. The plastic trough containing 200 ml of rearing water with solvent served as the control. Dead larvae and pupae was removed and counted at 24 h intervals. Observations on larval and pupal mortality were recorded. The experiment was replicated five times. Percentage mortality observed in the control was subtracted from that observed in the treatments (Abbot, 1925). The day from moulting of the larvae to pupation and to adulthood was noted. Fecundity was assessed by counting the number of eggs laid during the life span by control and experimental mosquitoes. The larvae and pupal duration of treated and control individuals were compared and developmental rates were determined.

2.4 Adulticidal assay

Culex quinquefasciatus fresh adults were exposing to filter paper treated with different concentration of plant extract. The paper was keep inside the beaker. Muslin cloth covering the beaker was also treated. Control insects were exposed only to distilled water with solvent treated paper and muslin cloth. Mortality count was taken after 24h (Sharma *et al*., 1992).

2.5 Ovipositional assay

Different quantities of plant extract from a stock solution were mixed thoroughly with 200 ml of rearing food in 250 ml glass jars to obtain the concentration desired for the tests with *Culex quinquefasciatus*. The gravid females were given a choice between treated and control jars. During the tests, the groups of females were kept separate for 48 h in cages measuring 25 x 25 x 30cm. After the eggs were counted the oviposition activity index (OAI) was calculated using the formula:

$$OAI = \frac{(Nc-Nt)}{(Nc+Nt)} \times 100$$

Where Nc is the number of eggs in the control

Nt is the number of eggs in the treatment

2.6 Ovicidal assay

Culex quinquefasciatus eggs were released in water. The test extracts were added in desired quantities and hatching were observed for one week. The eggs were then exposed to deoxygenated water and the numbers of hatching eggs were recorded. Percentage hatching was compared with the control in which only distilled water with solvent were used (Sharma *et al*., 1992).

2.7 Repellency activity

Different concentrations of plant extract were mixed thoroughly with 10ml of goat blood in glass plates. The untreated blood served as the control. Adult females were release into each cage. The number of females landing on the treated blood and untreated blood were record. The repellent index of the plant extracts were calculated as described by (Murugan and Jeyabalan, 1999).

2.8 Biting deterrency activity

The percentage protection in relation to dose method was used (WHO, 1996). Blood starved female *Culex quinquefasciatus* (100 nos), 3 - 4 days old, was kept in a net cage (45x30x45 cm^2). The arm of the test person was cleaned with isopropanol. After air drying the arm, a 25 mc^2 area of the dorsal side of the skin was exposed, the remaining portion was covered by rubber gloves. The plant extracts were dissolved in ethanol, where distilled water with solvent served as control. Different concentration of the plant extracts was applied. The control and treated arms was introduced simultaneously into the cage. The numbers of bites was count over 5 minute from 6 pm to 6 am. The experiment was conducted five times. The percentage protection was calculated by using formula:

$$\text{Percentage protection} = \frac{(\text{No. of bites received by control arm}) - (\text{No. of bites received by treated arm})}{(\text{No. of bites received by control arm})}$$

2.12 Statistical analysis

All data was subject to analysis of variance and the treatment mean was separated by Duncan's Multiple Range Test (Duncan, 1955). Statistical analysis was carried out using the (Statistical Package Social Science) SPSS software, version 16.0.

3. RESULTS

The ethanolic leaf extract of *Artemisia arborescens* exhibited a concentration-dependent larvicidal activity against all four larval instars of *Culex quinquefasciatus* (Table 1). No mortality was recorded in the control group across all instars. At 0.5% concentration, larval mortality was lowest, ranging from 45% in 1st instar, 41% in 2nd instar, 38% in 3rd instar, and 33% in 4th instar, indicating limited but measurable susceptibility at lower doses. At 1% concentration, the mortality notably increased, with 66%, 60%, 55%, and 51% mortality for 1st to 4th instars, respectively. This demonstrates enhanced toxicity with increasing concentration. At 2% concentration, the larvicidal effect was highest across all instars. The 1st instar showed 100% mortality, followed by 96% in 2nd instar, 91% in 3rd instar, and 85% in 4th instar. The increasing tolerance in later instars was evident, but high mortality still indicated strong bioefficacy of the extract. Overall, 1st instar larvae were the most susceptible, while the 4th instar exhibited the highest tolerance. The results confirm that *A. arborescens* ethanolic extracts possess significant larvicidal properties, which intensify with increasing concentrations and vary with larval developmental stage.

Table 1. Effect of ethanol leaf extracts of *Artemisia arborescens* on the larval mortality of *Culex quinquefasciatus*

Treatment	Concentration (%)	1st Instar (%)	2nd Instar (%)	3rd Instar (%)	4th Instar (%)
Control	00	00[d]	00[d]	00[d]	00[d]
Artemisia arborescens	0.5	45[c]	41[c]	38[c]	33[c]
	1	66[b]	60[b]	55[b]	51[b]
	2	100[a]	96[a]	91[a]	85[a]

Within a column means followed by the same letters are not significantly different at 5%level by DMRT.

Table 2. Effect of ethanol leaf extracts of *Artemisia arborescens* on the pupal mortality, adult mortality and adult emergence of Culex *quinquefasciatus*

Treatment	Concentration (%)	Pupal mortality (%)	Adult mortality (%)	Adult emergence (%)
Control	00	00[d]	00[d]	100[a]
Artemisia arborescens	0.5	35[c]	30[c]	79[b]
	1	60[b]	55[b]	65[c]
	2	86[a]	79[a]	52[d]

Within a column means followed by the same letters are not significantly different at 5%level by DMRT.

The ethanolic leaf extract of *Artemisia arborescens* demonstrated a marked concentration-dependent effect on the pupal and adult stages of *Culex quinquefasciatus* (Table 2). No pupal mortality, adult mortality, or reduction in adult emergence was observed in the untreated control group. At 0.5% concentration, moderate pupal and adult mortality were recorded, with 35% pupal mortality and 30% adult mortality, accompanied by 79% reduction in adult emergence. This indicates that even the lowest concentration interferes significantly with mosquito development. At 1% concentration, the toxicity increased substantially. Pupal mortality rose to 60%, adult mortality reached 55%, and adult emergence decreased sharply to 65%, demonstrating enhanced inhibitory effects on both pupal survival and subsequent adult formation. At 2% concentration, the extract produced the strongest impact. Pupal mortality reached 86%, adult mortality was 79%, and 52% of adult emergence was achieved, indicating complete blockage of adult mosquito development at this concentration. Overall, the results reveal that *A. arborescens* ethanolic extract is highly effective against the pupal and adult stages of *C. quinquefasciatus*, with efficiency increasing proportionally to the concentration. The extract's ability to completely prevent adult emergence at higher doses highlights its strong potential as a mosquito growth regulator and developmental inhibitor.

The ethanolic leaf extract of *Artemisia arborescens* produced notable adult repellent activity and strong ovipositional deterrence against *Culex quinquefasciatus*, with both effects increasing proportionally to concentration (Table 3). No repellency or oviposition deterrence was observed in the control group. At 0.5% concentration, the extract exhibited moderate adult repellency of 25% and oviposition deterrency of 31%, indicating the beginning of behavioral interference at lower doses. At 1% concentration, the repellent effect strengthened considerably, with adult repellency reaching 55%. Similarly, oviposition deterrency increased to 53%, demonstrating that gravid females increasingly avoided treated sites. At 2% concentration, the extract produced the highest behavioral impact, with 76% adult repellency and 81% ovipositional deterrency, showing strong avoidance by adult mosquitoes and a substantial inhibition of egg-laying activity. Overall, the ethanolic extract of *A. arborescens* exhibited potent adult repellent and oviposition-deterrent properties. The higher concentrations were particularly effective, suggesting that the plant extract can significantly reduce mosquito–human contact and interrupt reproductive cycles through strong behavioral modifications.

Table 3. Effect of ethanol leaf extracts of *Artemisia arborescens* on adult repellency and ovipositional deterrency of *Culex quinquefasciatus*

Treatment	Concentration (%)	Adult Repellency (%)	Oviposition Deterrency (%)
Control	00	00^d	00^d
Artemisia arborescens	0.5	25^c	31^c
	1	55^b	53^b
	2	76^a	81^a

Within a column means followed by the same letters are not significantly different at 5%level by DMRT.

Table 4. Larval duration of *Culex quinquefasciatus* after the treatment of ethanol leaf extracts of *Artemisia arborescens*

Treatment	Concentration (%)	1st Instar (days)	2nd Instar (days)	3rd Instar (days)	4th Instar (days)
Control	00	1.6^d	2.6^d	3.8^d	4.3^d
Artemisia arborescens	0.5	3.4^c	4.5^c	5.2^c	6.5^c
	1	5.4^b	6.7^b	7.4^b	8.6^b
	2	7.3^a	9.5^a	9.2^a	10.2^a

Within a column means followed by the same letters are not significantly different at 5%level by DMRT.

The larval developmental period of *Culex quinquefasciatus* was significantly prolonged following treatment with ethanol leaf extracts of *Artemisia arborescens* (Table 4). In the control group, the mean duration of the 1st, 2nd, 3rd , and 4th instars was 1.6, 2.6, 3.8, and 4.3 days, respectively, representing the normal rate of larval development. Treatment with 0.5% concentration caused a noticeable delay at each developmental stage. The 1st instar increased to 3.4 days, the 2nd instar to 4.5 days, the 3rd instar to 5.2 days, and the 4th instar to 6.5 days, indicating early disruption of growth and molting processes. At 1% concentration, the delay became more distinct, with larval durations extending to 5.4, 6.7, 7.4, and 8.6 days for the 1st to 4th instars, respectively. This shows stronger physiological impairment of larval development. At the highest concentration of 2%, the extract caused the maximum extension of larval duration. The 1st instar required 7.3 days, the 2nd instar 9.5 days, the 3rd instar 9.2 days, and the 4th instar 10.2 days, nearly doubling or tripling the normal developmental time. Overall, the results clearly show that *A. arborescens* ethanol extract induces a dose-dependent extension of larval developmental periods. The prolonged larval duration suggests interference with growth regulation, molting hormones, and metabolic activity, ultimately contributing to reduced survival and delayed progression to the pupal stage.

Table 5. Pupal and adult duration of *Culex quinquefasciatus* after the treatment of ethanol leaf extracts of *Artemisia arborescens*

Treatment	Concentration (%)	Total pupal duration (days)	Total adult duration (days)
Control	00	3.6^d	58^a
Artemisia arborescens	0.5	6.2^c	42^b
	1	8.5^b	31^c
	2	10.6^a	24^d

Within a column means followed by the same letters are not significantly different at 5%level by DMRT.

The ethanolic leaf extract of *Artemisia arborescens* produced marked alterations in both pupal and adult developmental durations of *Culex quinquefasciatus* (Table 5). In the untreated control group, the total pupal duration was 3.6 days, and the adult longevity was 58 days, representing normal developmental and survival patterns. At 0.5% concentration, the pupal duration was extended to 6.2 days, while the adult duration decreased significantly to 42 days. This indicates early developmental disruption coupled with reduced adult survival. At 1% concentration, the effects became more pronounced. Pupal duration increased further to 8.5 days, while adult longevity declined to 31 days, suggesting stronger physiological stress and reduced fitness among emerging adults. Treatment with the highest concentration (2%) resulted in the greatest impact. The pupal duration nearly tripled to 10.6 days, and the adult lifespan was reduced to 24 days, representing the shortest survival recorded. This demonstrates severe impairment in growth and adult viability at elevated doses. Overall, the results show a clear concentration-dependent trend: *A. arborescens* extract prolongs pupal development while reducing adult lifespan. Such dual effects reduce population survival and reproductive potential, reinforcing the plant's potential as an effective mosquito developmental inhibitor.

Table 6. Effect of ethanol leaf extract of *Artemisia arborescens* on fecundity and egg hatchability of *Culex quinquefasciatus*

Treatment	Concentration (%)	Fecundity (No. of eggs)	Eggs hatchability (%)
Control	00	205[a]	100[a]
Artemisia arborescens	0.5	142[b]	62[b]
	1	112[c]	41[c]
	2	86[d]	28[d]

Within a column means followed by the same letters are not significantly different at 5%level by DMRT.

The ethanolic leaf extract of *Artemisia arborescens* significantly influenced both the fecundity and egg hatchability of *Culex quinquefasciatus*, with effects increasing proportionally to concentration (Table 6). In the control group, female mosquitoes laid an average of 205 eggs, with 100% egg hatchability, representing normal reproductive output. At 0.5% concentration, fecundity decreased to 142 eggs, and egg hatchability was reduced to 62%, indicating the onset of reproductive suppression. This suggests that the extract interferes with egg development and embryonic viability even at low concentrations. At 1% concentration, stronger inhibitory effects were observed. Fecundity declined further to 112 eggs, while hatchability dropped sharply to 41%, demonstrating substantial impairment of reproductive physiology and egg survival. At the highest dose of 2%, the extract caused the most pronounced reduction. Fecundity decreased to 86 eggs, reflecting a significant decline in oviposition rate, and egg hatchability was reduced to 28%, indicating severe embryonic lethality or developmental arrest. Overall, *A. arborescens* ethanol extract exerted a strong dose-dependent reduction in both the number of eggs produced and their subsequent hatchability. The marked decline in fecundity and egg viability highlights the extract's effectiveness in disrupting mosquito reproduction, thereby contributing to long-term population suppression.

Table 7. Effect of ethanol leaf extract of *Artemisia arborescens* on Larval-pupal intermediate of *Culex quinquefasciatus*

Treatment	Concentration (%)	Larval-pupal Intermediate (%)
Control	00	00[d]
Artemisia arborescens	0.5	34[c]
	1	52[b]
	2	75[a]

Within a column means followed by the same letters are not significantly different at 5%level by DMRT.

The formation of larval–pupal intermediates in *Culex quinquefasciatus* increased markedly following treatment with ethanol leaf extracts of *Artemisia arborescens* (Table 7). No intermediate forms were observed in the control group (0%), indicating normal and uninterrupted metamorphosis under untreated conditions. At 0.5% concentration, intermediate formation was recorded at 34%, demonstrating the initial onset of developmental disruption. The presence of malformed or intermediate stages suggests interference with hormonal regulation, particularly ecdysis and molting processes. At 1% concentration, the percentage of larval–pupal intermediates increased substantially to 52%, indicating a higher level of physiological stress and incomplete metamorphosis. The highest dose (2%) resulted in the maximum proportion of intermediates (75%), showing severe impairment of the larval–pupal transition. Such high rates of intermediate formation indicate strong toxicity of the plant extract toward key developmental pathways. Overall, the extract exhibited a strong dose-dependent effect, with increasing concentrations of *A. arborescens* causing progressively greater disruption of normal metamorphosis. The formation of larval–pupal intermediates reflects interference with endocrine regulation and developmental processes, ultimately contributing to reduced survival and population suppression.

Table 8. Effect of ethanol leaf extract of *Artemisia arborescens* on biting deterrency of *Culex quinquefasciatus*

Treatment	Concentration (%)	Biting deterrency (%)
Control	00	00[d]
Artemisia arborescens	0.5	30[c]
	1	55[b]
	2	76[a]

Within a column means followed by the same letters are not significantly different at 5%level by DMRT.

The ethanol leaf extract of *Artemisia arborescens* showed a marked biting deterrent effect against *Culex quinquefasciatus* (Table 8). In the untreated control group, no biting deterrence was observed (0%). However, the application of plant extract produced progressively higher deterrent activity with increasing concentrations. At 0.5% concentration, the extract exhibited a moderate biting deterrence of 30%, indicating partial repellency. A further increase to 1% concentration resulted in a substantial rise in deterrence, reaching 55%. The highest concentration tested (2%) demonstrated the maximum biting deterrence of 76%, showing strong protective efficacy. Overall, the results indicate a clear dose-dependent increase in the biting deterrent activity of *A. arborescens* ethanol leaf extract against *Cx. quinquefasciatus*, highlighting its potential as a plant-based mosquito deterrent.

4. DISCUSSION

The use of insecticides is a major control measure for eradicating *Culex quinquefasciatus* mosquitoes. Similarly, a vital means to control the dengue fever epidemic is by killing both the larvae and adult *Culex quinquefasciatus* mosquitoes using insecticides (Aljameeli, 2023). However, it is now well known insecticides are dangerous due to a number of reasons, such as the emergence of insecticide resistance, which causes pest resurgence, the disruption of ecosystems, concerns for human health and the environment, and their toxicity to non-target organisms (Boukraa *et al*., 2022). As a result, considering their efficacy and safety, plant-based bioinsecticides have become more popular in recent years as alternatives. The use of isolated compounds, Eos inclusive, is beneficial owing to the possibility of their synthesis, enabling the possibility of obtaining them in large quantities necessary for practical use (Luz *et al*., 2020).

Lippia javanica was the only plants investigate in terms of its general constituents and the isolation of bioactive compounds responsible for toxicity, insecticidal, and repellency activity against mosquitoes in Zimbabwe (Chagonda and Chalchat, 2015). The major constituents were 1,3,5 cycloheptatriene, alphapinene, and eucalyptol while the other phytochemicals were caryophyllene, carvone, linalool, ocimenone, sabinene, piperitenone ,tagetenone, ipsenone, myrcenone, ipsdienone, and p-cymene (Chagonda and Chalchat, 2015). The phytochemistry of other mosquito-repellent plants needs to be evaluated as well. Secondary metabolites such as essential oils, alkaloids, phenols, terpenoids, steroids, and phenolics have been isolated from different plant extracts and reported for their mosquitocidal activities in studies conducted in other parts of the world (Bett *et al*., 2022).

However, despite their promising activities, establishing a complex mixture of essential oils as a viable insecticide is challenging, making it unlikely, from a commercial standpoint, that any single EO will be utilized for insect control without further research. Several factors must be considered, such as the availability of raw materials, the preparation and formulation of bioinsecticides with controlled chemical compositions, and the identification of active constituents before labeling an EO as a potential agent against mosquitoes (Isman, 2020; Hematpoor *et al*., 2017). Isman (2020) recently discussed the development of a bioinsecticide meeting commercial demands, emphasizing the importance of defining procedures for formulating insecticidal products. Nanotechnology holds promise for enhancing the stability and bioavailability of essential oils in these formulations, ensuring the persistence of volatile compounds and facilitating delivery through insect skin. Despite challenges in biomass availability, particularly regarding plant species cultivation, emerging alternatives are being explored to address this issue. However, the selection of an EO or its bioactive components requires further investigation for resolution, given the inherent challenges associated with each option.

Besides documenting its mosquito-repellent effect, studies have been conducted to evaluate its larvicidal effect under laboratory conditions. Eukubay *et al*. (2020) evaluated the methanol leaf extract of *C. aurea* at concentrations ranging from 50 to 300 ppm against 3rd instar *An. Arabiensis* larvae and found 100% mortality at the maximum concentration. Similarly, (Muhammed *et al*., 2024) assessed the larvicidal effects of aqueous, hexane, and methanol crude leaf extracts of *C. aurea* with various concentrations ranging from 25 ppm to 300 ppm against the third to fourth instar larvae of *An. Stephensi*. The authors reported that 100% mortality was found in aqueous and methanol extracts with high concentrations (200-300 ppm). Such a high biological activity might be attributed to the presence of bioactive secondary metabolites, including phenolic, tannin, alkaloids, flavonoids, and saponin (Kemal *et al*., 2013).

In this context, the scarcity of literature data regarding the biological activity of *Aloysia citrodora* and *Artemesia arborescens* plants against adult *Culex quinquefasciatus* underscores the importance of investigating the properties and effectiveness of this plant essential oil for vector control purposes. The results of our study demonstrate that the essential oil derived from this plant is effective in eliminating *Culex quinquefasciatus* in the larval and adult stages. After obtaining interesting results from experimental analyses on the larvicidal and adulticidal activities of *Aloysia citrodora* and *Artemesia arborescens* essential oil, a molecular docking analysis was conducted between key enzymes of *Culex quinquefasciatus* species and the three main compounds of the essential oil.

The control of immature (aquatic) stages of mosquitoes through source reduction, application of insecticides or through combination of both approaches is a preferred strategy for controlling mosquito population (Mullai *et al*., 2007). Synthetic insecticides are not environment friendly; therefore researchers are now looking for plant-based insecticides to control mosquitoes (Shaalan *et al*., 2005). The insecticidal potential of *Aloysia citrodora* and *Artemesia arborescens* whole-plant extract was investigated against *Cx. quinquefasciatus.* The as *Aloysia citrodora* and *Artemesia arborescens* non-polar extract showed remarkable larvicidal and pupicidal activity against *Cx. quinquefasciatus*. The larvicidal and pupicidal activity of *Aloysia citrodora* and *Artemesia arborescens* whole-plant extract was positively correlated with increase in extract concentration. Such correlations have also been reported by other researchers (Adhikari *et al*., 2012; Rawani *et al*., 2013), In this research, the extract resulted in higher mortality of 2nd instar larvae (LC_{50}=324.6 ppm) as compared to the mortality of 4th instar larvae (LC_{50}=495.6 ppm). According to (Andrande and Modolo, 1991), the higher susceptibility of early instar larvae to the extract solution is due to higher filtration rate in early instars. Chowdhury *et al*. (2009), Kovendan *et al*. (2012) also reported that early instars larvae are more susceptible to insecticidal agents than late instar larvae when exposed. During this study, it was also observed that mosquito pupae are less susceptible to *Aloysia citrodora and Artemesia arborescens* extract than larvae.

Brummer expression was found to be the most abundant in Drosophila embryos and adults, with negligible expression in 1st and 2nd instar larvae (Grönke *et al*., 2005). Studies by dos Santos corroborated those findings when Bmm expression was compared across larvae through adult mosquitoes; increased expression of Bmm correlated with decreased amounts of TG in these mosquito life stages (dos Santos *et al*., 2024). It is reasonable that TGs sequestered in *An. Stephensi* eggs are rapidly hydrolysed by the Bmm homolog to fuel hatching. Low Bmm expression in 1st and 2nd instar larvae (as shown in both Drosophila and *Ae. aegypti*) would permit rapid replenishment of TGs as larvae feed. Thus the coordinated use, rebalancing and accumulation of TGs as mosquitoes develop may be a universal metabolic mechanism shared across mosquito families and other insect species with similar life cycles. Two LysoPCs were identified as increased lipids in 1st instar versus 2nd instar larvae, which may indicate increased endogenous cholesterol biogenesis upon hatching (Clayton *et al*., 1964, Clifton and Noriega, 2012). Finally, we found that crustecdysone (20-hydroxyecdysone, 20E) is present in a greater abundance in all four instar larvae stages and the pupation stage, versus other stages. *Crustecdysone* is a key mosquito hormone regulating several processes throughout the mosquito life cycle, including moulting, insecticide resistance, and fecundity (Ekoka *et al*., 2021).Previously, 20E agonists were shown to be effective insecticides in *An. gambiae*, *Aedes aegypti*, and other mosquito larvae (Beckage *et al*., 2004). Levels of 20E were also shown to increase as *Ae. Aegypti* L4 larvae ecdysis to pupae (Margam *et al*., 2006). Interestingly, these previous studies, exploring both 20E's importance in moulting (Margam *et al*., 2006) and as a target for novel insecticides (Beckage *et al*., 2004) were conducted on 4th and 3rd instar larvae, respectively. Our studies suggest that 20E may play additional mosquito maturation roles and its agonists maybe equally or more effective on newly hatched eggs,as this hormone is abundant in all larval stages.

Also, treatment of *C. antennatus* third larval instar with tested extracts resulted in prolongation in both larval and pupal durations as compared with control congers; the prolongation in larval and pupal periods was solvent and concentration-dependent and was similar to that reported by Sharma *et al*. (2011) using petroleum ether extract of *Artemisia annua* against *A. stephensi* and *C. quinquefasciatus* larvae using ethanolic extract of *Melia azedarach* leaves on *Ae. Aegypti* larvae using *Moringa oleifera lectin* against *Ae. aegypti* larvae (Hassanali *et al*., 2019) using petroleum ether extract from leaves of *L. camara* against larvae of *A. multicolor* (Shehata, 2020) using methanol, chloroform, and petroleum ether extracts from leaves of *Pr. Domestica* and *Rh. Cathartica* against *C. pipiens* (Shahat *et al*., 2020) using *Otostegia fruticosa* leaves extracts against *C. pipiens* and (Shahata *et al*., 2020) using *Py. Communis* PCH and PCM extracts against *A. pharoensis*. In addition, the toxic effect of tested extracts extended to *C. antennatus* pupae depending on the solvent used in the extraction and the concentration of the extract. Pupal mortality percentages recorded in the present study confirm those reported by (Asiry *et al*., 2017) using ethanolic leaf extracts of *Citrullus colocynthis, Artemisia annua, Pergularia tomentosa* and *Rhanterium epapposum* against *Ae. aegypti,* (Shahat, 2020) using methanol, chloroform, and petroleum ether extracts from leaves of *Pr. domestica* and *Rh. cathartica* against *C. pipiens,* (Shahat *et al*., 2020) using *O. fruticosa* leaves extracts against *C. pipiens* (Shahat *et al*., 2020) using *Py. communis* PCH and methanol PCM extracts against *An. pharoensis.*

In the present study treatment with plant extracts exhibited extension of total developmental period significantly when compared with control. Growth regulatory effect in mosquitoes is normally caused by hormonal modulations as in other insects and cause either delaying or shortening their larval/pupal durations and lead to malformations and death (Mullai, *et al*., 2008). As Juvenile hormone is a key regulator of development and reproduction in mosquitoes (Zhu and Noriega, 2016), the activity of the selected fractions may be attributed to affecting the juvenile hormone titre which may result in the extension of larval duration and induces incidence of malformations.

Previous studies have confirmed that spermathecae, which promote sperm maturation and capacitation, have an extremely strong correlation with female fertility and fecundity (Allen and Spradling, 2008). Following copulation, sperm along with a mixture of seminal fluid move into spermathecae, where they are subsequently maintained and stored (Durant and Donini, 2020). Thus, the composition and physiological conditions of spermathecae undergo dynamic changes to ensure sperm survival. Microtubules play indispensable roles in Sertoli and germ cell development, which may be the reason for the upregulation of proteins enriched with these GO terms after insemination (Helmke *et al*., 2013). Moreover, the mating-induced metabolic and glycolysis/gluconeogenesis regulation reflect that females provide ATP for sperm motility and survival during storage, which is consistent with previous studies in mice, Drosophila, and honeybees (Storey, 2008).

The adult which emerged from treated larvae showed a great reduction in fecundity. The same results were mentioned against some insects (Deore and Khadabadi, 2009). Many reports showed changes in fecundity after treatment with biopesticide, *B. thuringiensis* (Aissaoui *et al*., 2023*)*. They discovered that the extract from reduced the number of female eggs and killed mosquito larvae (100% mortality). Similarly, *Aloysia citrodora* and *Artemesia arborescens* leaf extracts were found to be acutely toxic at 212.1 mg/l and chronically toxic at 144.2 mg/l by Zaitoun *et al*. (2009) also who referred to the ability of extracts on inhibit adult emergence and egg hatchability.

As an attractive point, the descending concentration gradient leads to a descending rate of oviposition and hatching rates. At high concentrations of extract, the oviposition rate recorded its maximum value, and more than 50% of the total eggs were laid. Similarly, oviposition attitude was observed at moderate concentration, and less than 31% of the total eggs were counted. In agreement, the use of a very low concentration of *A. nilotica* showed a noticeable downfall of the number of eggs laid to less than 13% and 5% of the total eggs laid for the concentrations 250 ppm and zero (control without extract), respectively, confirming the direct relationship and oviposition rate. Moreover, the hatching rate is inconsistent with the oviposition rate. The hatchability rates were affected by the concentration gradient, and at zero concentration, the hatching ability was raised to 95% of the eggs laid at this concentration, whereas the higher concentration revealed a lower hatchability rate, confirming the inverse relationship between the concentration and the hatching rate. Based on the experimental data, the concentration of extract is directly proportional to oviposition rate and inversely proportional to hatching ability, which may introduce a reasonable interpretation for the dual benefit of acacia. Through what some active ingredients do to attract insects (Conchou *et al*., 2019; Spanoudis *et al*., 2022), perhaps through extracting odorant chemical code, at the same time, the rest of the active ingredients help kill the larvae and reduce the chances of eggs hatching (Ahmad *et al*., 2021).

In the present study the solvents extract of *Artemisia vulgaris* and *Salvia leucantha* plants is also oviposition deterrent against mosquitoes. The gravid female adults of *Cx. quinquefasciatus* mosquito were mostly avoidingthe containers for egg laying which were containing *Artemisia vulgaris* and *Salvia leucantha* whole-plant. Highest concentration of extract solution showed maximum oviposition deterrence. The deterrence of oviposition was presented in percent effective repellence (% ER) and oviposition activity index (OAI). Negative OAl values were noted for the containers containing extract solution. According to (Govindarajan *et al*., 2011), negative OAls values indicate oviposition deterrence. Few studies have been conducted on the oviposition deterrent activity of medicinal plants against mosquitoes (Elimam *et al*., 2009; Kamaraj *et al*., 2009; Prathibha *et al*., 2014; Reegan *et al*., 2015). Some active principles contained in plant extracts act as chemosterilent or growth regulators while some create olfactory stimuli that cause repellence (Prathibha *et al*., 2014). Most of the insect repellent plants are oviposition deterrent (Mehra and Hiradhar, 2002; Rajkumar and Jebanesan, 2009).

Rajkumar and Jebanesan (2009) proved that the leaf extract of *C. asiatica* has larvicidal properties and is an inhibitor for adult emergence against *C. quinquefasciatus.* The effects of the tested extract, adult emergence, and adulticidal activity of the mosquitoes are remarkably greater than those reported for other plant extracts in the literature. For example at the highest concentration, 50% inhibition of the emergence of the adult mosquitoes was observed by the use of the ethyl acetate fractions of *Calophyllum inophyllum* seed and leaf, *Solanum suratense* and *Samadera indica* leaf extracts, and the petrol ether fraction of *Rhinocanthus nasutus* leaf extract on *C. quinquefasciatus*, *A. stephensi*, and *A. aegypti* (Muthukrishnan *et al*., 1999). The above findings support the results observed in this study the plant *Aloysia citrodora* and *Artemesia arborescens* act as potent larvicide, adulticide as well as disrupting the growth of larvae of *Culex quinquefasciatus.*

Herbal mosquito repellents offer a sustainable and eco-friendly alternative to synthetic insecticides, which may have adverse effects on the environment and human health. By harnessing the repellent properties of medicinal plants, herbal formulations can reduce reliance on chemical pesticides and contribute to environmentally sustainable mosquito control practices. Additionally, cultivating and harvesting medicinal plants for repellent production can provide economic opportunities for local communities, supporting livelihoods and biodiversity conservation (Govindarajan *et al*., 2011).

The repellent properties of various plant essential oils (EO) are linked to the presence of monoterpenoids and sesquiterpenes (Benelli *et al*., 2018). These compounds are found to be main elements present in *M. communis* EO in our analysis (Yezli *et al*., 2024). Monoterpenes like a-pinene, limonene, terpinolene, citronellol, citronellal, camphor and thymol are commonly found in numerous essential oils known for their mosquito repellent activity (De Souza *et al*., 2019). Additionally, natural compounds and plant derivatives contribute to another significant bioactivity in mosquito repellence. Plants containing monoterpenes can alter biochemical and physiological insect activities (Waliwitiya *et al*., 2009). The repellent actions of these compounds offer promising alternatives for the control and protection against these mosquitoes (De Souza *et al*., 2019).

The leaf was the main part of the plant used in the preparation of repellents in most of the studies and most of the plants were from families rich in essential oils. The prominent application of leaves for most repellent plants is due to the readily available volatile compounds in leaves (Youmsi *et al*., 2017). Different concentrations of extracts were used in all the studies that *Culex* *quinquefasciatus* were used as test mosquitoes complicating the interpretations of the results. There is a need for standardization of the concentrations of extracts from different plants that are evaluated using one mosquito species so that results are comparable.

Herbal derivatives from mosquito repellent plants used in these studies were effective for shorter periods when compared to synthetic repellents which are known to provide up to 8 hours or more of protection. The short protection periods can be attributed to the high volatility of essential oils. The protection period of herbal derivatives can be enhanced through the development of formulations that extend the duration of the protection period. Only the extracts from *L. javanica* were comparable to synthetic repellents since they provided protection from *Culex quinquefasciatus* biting for 8 hours. The phytochemical constituents of *L. javanica* have known pharmacological properties for mosquito repellency (Changonda and Chalchat, 2015).

The mode of action of plant-based repellents can vary depending on the specific plant species and their bioactive constituents. While some plants may primarily act through olfactory interference, others may exert their repellent effects through multiple mechanisms, including both sensory disruption and physiological deterrence. This variability underscores the complex nature of plant-based repellency and the need for further research to elucidate specific mechanisms for different plant species (Sukumar *et al*., 1991).

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