



# EVALUATION OF *LITSEA FLORIBUNDA* LEAF EXTRACT ON MOSQUITOCIDAL POTENTIAL AGAINST FILARIAL VECTOR, *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

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## Abstract:

In the present study the acetone extract of *Litsea floribunda* leaves was evaluated against *C. quinquefasciatus*. *Litsea floribunda* leaves extract was tested for their biological, larvicidal, pupicidal, adulticidal, antiovipositional activity, repellency and biting deterrence against *C. quinquefasciatus*. The larval and pupal mortality increased significantly with increasing the concentrations levels of *Litsea floribunda* leaves extract. The adult emergence also significantly affected by the treatment of *Litsea floribunda* leaves extract. The larval and pupal duration were extended by the treatment of *Litsea floribunda* leaves extract. Adult longevity and fecundity were greatly reduced after the treatment. At 2% the *Litsea floribunda* leaves extract showed strong ovipositional deterrence. The *Litsea floribunda* leaves extract treatment significantly enhanced the repellency and biting deterrence against *C. quinquefasciatus*.

## Key Words

*Culex quinquefasciatus*, *Litsea floribunda*, mortality, larvicidal, pupicidal, adulticidal, repellency, deterrence

## 1. Introduction

Mosquitoes are the most important group of blood sucking insects that cause nuisance and transmit diseases to humans and other warm blooded animals. The nuisance and annoyance caused by mosquitoes is not easy to translate into economic value, however it is as vectors of disease that mosquitoes are most often of concern. Vector mosquitoes and the parasites and pathogens that they transmit, are recognised to have played an important role in the development and dispersal of the human race, being responsible for some events that have shaped the course of history. Although vaccines, chemoprophylaxis, chemotherapy, genetics and vector control measures are becoming more sophisticated, even now, none of the major mosquito-borne diseases of the world can be said to be under complete control.

*Culex quinquefasciatus* is a markedly domestic species. The adult females bite people and animals throughout the night, indoors and outdoors. During the day they are inactive and are often found resting in dark corners of rooms, shelters and culverts. They also rest outdoors on vegetation and in holes in trees in forest areas. Female mosquitoes feed on animals and humans. Most species show a preference for certain animals or for humans. They are attracted by the body odours, carbon dioxide and heat emitted from the animal or person. Some species prefer biting at certain hours, for example at dusk and dawn or in the middle of the night. Feeding usually takes place during the night but daytime biting also occurs. Some species prefer to feed in forests, some outside of houses, and others indoors. Because digestion of the blood-meal and development of the eggs takes several days, a blood-fed mosquito looks for a safe resting place that is shaded and offers protection from desiccation. Some species prefer to rest in houses or cattle sheds, while others prefer to rest outdoors, on vegetation or at other natural sites. Mosquitoes do not usually bite while eggs are developing. The behaviour of mosquitoes determines whether they are important as nuisance insects or vectors of disease, and governs the selection of control methods. Species that prefer to feed on animals are usually not very effective in transmitting diseases from person to person. Those that bite in the early evening may be more difficult to avoid than species that feed at night. Mosquitoes that rest indoors are the easiest to control.

About 550 species of *Culex* have been described, most of them from tropical and subtropical regions. Some species are important as vectors of bancroftian filariasis and arboviral diseases, such as Japanese encephalitis. In some areas they are a considerable nuisance. Rafts of 100 or more eggs are laid on the water surface. The rafts remain afloat until hatching occurs 2–3 days later. *Culex* species breed in a large variety of still waters, ranging from artificial containers and catchment basins of drainage systems to large bodies of permanent water. The most common species, *Culex quinquefasciatus*, a major nuisance and vector of bancroftian filariasis, breeds especially in water polluted with organic material, such as refuse and excreta or rotting plants. Examples of such breeding sites are soakaway pits, septic tanks, pit latrines, blocked drains, canals and abandoned wells. In many developing countries *Culex quinquefasciatus* is common in rapidly expanding urban areas where drainage and sanitation are inadequate.

*C. quinquefasciatus* is a vector of lymphatic filariasis (tropical disease) which accounted 120 million people infected worldwide, and 44 million people having common chronic manifestation. According to World Health Organization (WHO) estimates, globally about 90 million people are infected with *W. bancrofti* and ten times more peoples are at the risk of being infected. So far, 25 million people harboring microfilaria and 19 million people suffering from filarial disease manifestations were recorded from India.

Mosquitoes are the most important group of bloodsucking arthropods. They not only create nuisance to humans by biting, but also transmit serious diseases with many socioeconomic consequences. The situation has become worse over the last decade due to global climatic changes. This, in addition to other factors has favored them to adapt to a wide range of habitats and thereby to increase its population in many parts of the world (Michaelakis *et al.*, 2009). Several mosquito species belonging to the genera *Anopheles*, *Culex* and *Aedes* are acting as vectors for many pathogenic organisms causing diseases like Malaria, Filariasis, Japanese Encephalitis, Dengue fever, Yellow fever etc. These diseases spread globally, causing high levels of human mortality and thereby acting as factors impeding the economic development of most of the developing countries across the world (Borah *et al.*, 2012). Various pesticides and chemical formulations have been employed in an effort to control or eradicate mosquito populations. Even though they are highly efficacious against the target species, these pesticides are facing threats due to the development of resistance to chemical insecticides by the mosquitoes, resulting in rebounding vectorial capacity (Senthilkumar *et al.*, 2008). The long-term stability of many of these pesticides and their tendency to bio accumulate in non-target organisms have fostered many environmental and human health concerns. Plant secondary metabolites are considered to be a potential alternative approach against different species of mosquitoes and their various immature stages due to their richness in bioactive compounds, easy availability, environmental safety etc. (Mohan *et al.*, 2009).

Current and past strategies for mosquito control are based on synthetic insecticides, such as dichlorodiphenyltrichloroethane (DDT), temephos, and Malathion. Although chemical agents have been successfully utilized in mosquitoes control during the last decades, the continuous use of these agents has resulted in the development of resistance, environmental impacts, and undesirable effects on non-target organisms, including humans (Deletre *et al.*, 2019). All these factors have created a need for new biodegradable and renewable alternative insecticides. In this context, the use of plant derived natural products for vector control has various attractive characteristics, including biodegradability, availability at affordable prices, smaller toxicity, and broad-spectrum target-specific activities against different mosquito species (Ghosh *et al.*, 2012).

Beside repellents are often used to protect against mosquitoes bite by applying on human skin (Perich *et al.*, 1994). The larvicidal effects of this solution were determined by WHO against different instars of *Culex quinquefasciatus*. The *Culex* genus of mosquitoes found everywhere and cause several health hazards to human (Nikkon *et al.*, 2011). It is found that the botanical phytochemicals are more potent alternative to synthetic insecticides. The flowers of Tagetes are helpful to protect ourselves from colds, coughs and ulcers. There are different effects of Tagetes like antibacterial, antifungal and cytotoxic effects (Jayaraman, 2015). There is a positive test of glycosides in ethanol extract and chloroform shows the presence of terpenoids and flavonoids. The crude ethanol extract shows more effectiveness against every instar of larvae of *Culex quinquefasciatus* (Nath *et al.*, 2006). The moiety of compound changes into more toxic substance in the larval alimentary canal, integument and it is a time dependent effect (Afzal *et al.*, 2018). Alpha terthienyl is a constituent of plants belonging to tagete species. The exposure of plant extract to fourth instar larvae of *Culex* shows high level toxicity under sunlight (Afzal *et al.*, 2018). The different oils were which have protection time of 8 hours and hundred percent repellency against the mosquito's larvae. Nematocidal work of roots refers to thienyls but essential oils that we obtained are terpenoids (Govindrajana *et al.*, 2012). There is a wonderful effect of UV in flourishing the effect of thienyl. Various species of Tagetes oil identified by gas chromatography were shown to contain limonene, oscimenene (Nath *et al.*, 2006). The effects of extract obtained from plants part (roots, flower and leaves) now become important.

The effect of extract is increased with concentration of oil. Vector control disease is stopped by using larvicidal recommended by WHO (Jayaraman, 2015). Essential oils are obtained by steam distillation. The cleavanger type apparatus is used for the extraction of essential oils. The more supplement of chemical larvicides will development of more resistance in vector (Amer and Mehlhorn, 2006). It is a great challenge to introduce chemical pesticides in environment. It is a main attention of researchers to find out the best natural pesticides. Essential oils contain low molecular terpenes and phenolics (Amer and Mehlhorn, 2006). The compounds show their effects on larvae by neurotoxic effects involving different mechanism. There is induction of sub-lethal effects by the use of synthetic larvicides (Jayaraman, 2015). The methanolic extract effect is more on the fourth instar larvae of *Cx. quinquefasciatus*.

The formation of natural pesticides is less expensive, shows more effect on larvae and moreover it is ecofriendly (Perich *et al.*, 1994). The obtained extract was used at different concentration and another simple method to extract out is first to dry the plant and to powder it. The acetone extracts of Tagetes showed growth inhibitory and juto the treated larvae. *Culex quinquefasciatus* is the main mosquito vector; it is a vector for different diseases like filariasis and 120 million people affected by this worldwide. Thus the higher mortality is shown after 72 h period. In recent years the natural larvicidal extracts gain the

popularity among the organic growers and environment conscious consumers. The essential oils obtained show the larvicidal, insecticidal as well as antifungal properties (Afzal *et al.*, 2018). People show their interest in new natural products that are environmentally safe and affordable. Recently two reports showed that Tagetes have strong biocidal effects on both larvae and adults. 4 thiophenes are the powerful mosquito larvicidal agent (Singh *et al.*, 1987).

Marigold extracts have many properties like phenylpropanoids, carotenoids, flavonoids, etc. Some Tagetes species shows the antioxidant properties. The level of thiophene rises up slowly and attains the peak value of 0.008 percent. The extract usually stored at 4 degree Celsius. This study shows the biocidal activities of Tagetes and *Mentha arvensis* (Amer and Mehlhorn, 2006). Essential oils from the tremendous plants have been used for the insects, etc. present in home and gardens. Tagetes plant is worldwide recognized for its medicinal values. The tested insecticides have sublethal effect on ovicidal activity, pupal duration and adult mortality (Amer and Mehlhorn, 2006).

Tagetes and *Mentha arvensis* have the larvicidal properties. The plants have the alkaloid that shows the toxic effect on larvae of mosquitoes. Petroleum ether extract of Tagetes shows the toxic effect on larvae of tagete (Amer and Mehlhorn, 2006). For determination LC<sub>50</sub> value, the exposure time is very important. These extract are mainly applied in stagnant water and this would applied the fertility of adults formed from larvae exposed to oil (Amer and Mehlhorn, 2006).

Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors. Essential oils of leaf and bark of *Cryptomeria japonica* demonstrated high larvicidal activity against *Aedes aegypti* (Diptera: Culicidae) larvae (Cheng *et al.*, 2003). Insecticidal activity of plant essential oils has been well-described by (Isman, 1999). Azadiractin, the active ingredient of neem has long been recognised for its mosquito larvicidal capability. The extracts of *Murraya koenigii*, *Coriandrum sativum*, *Ferula asafetida* and *Trigonella foenum graecum* were found to be effective and showed encouraging results against *Ae. aegypti* and *Culex* (Diptera: Culicidae) mosquito larvae (Desai, 2002). It is also reported that many compounds with insecticidal potential have been isolated from the genus Piper—Piperidine, isolated from *Piper nigrum* (black piper) has been found to be just as active against adjuki bean weevils as the pyrethroids (Mwangi and Mukiana, 1988). Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and have different activities observed by many researchers (Babu and Murugan *et al.*, 1998; Venketachalam and Jebasan., 2001).

Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors (Khanna *et al.*, 2011). However, more concerted efforts have been undertaken to make environment-friendly compounds viable for field use and for large scale vector control operations. Sukumar *et al.* (1991) reported 99 families, 276 genera and 346 species to have insecticidal properties. One of such natural plants is *Alchornea cordifolia* which is an important medicinal plant in African traditional medicine and much pharmacological research has been carried out into its antibacterial, antifungal, cytotoxic, hypotensive and antiprotozoal properties, as well as its anti-inflammatory activities, with significant positive results (Koomson and Oppong, 2018; Koomson *et al.*, 2018; Koomson, 2020). Found out the leaves, bark and roots of the plant was effective in controlling stored products insect pests through contact toxicity and repellency activities.

The use of botanical insecticides has a long history and their use is as a safer solution to control various types of insect pests. Naturally occurring chemicals extracted from plants act quickly (compared to biological control), degrade rapidly and usually have low mammalian toxicity (Ghosh *et al.*, 2012). Botanical insecticides affect various insects differently and can be classified into six groups: repellents, feeding deterrents/antifeedants, toxicants, growth retardants, chemosterilants and attractants (Rattan, 2010). *Vitex ovata* was chosen considering several species from this plant genus were known to have larvicidal properties (Chandrasekaran *et al.*, 2019). Leaf extract from *V. trifolia* L. consists of 89 phytochemical components, with the major component identified as eucalyptol, which is known as an insect repellent. *Vitex ovata*, also known as *V. rotundifolia* (Munir, 1987), possesses a biological compound named rotundial with potent repelling activity against *Ae. aegypti* (Watanabe *et al.*, 1995). Therefore, the present study was carried out to evaluate the mosquitocidal property of *Litsea floribunda* leaf extracts against the filarial vector, *C. quinquefasciatus*.

## 2. Materials and Methods

### 2.1 Preparation of *Litsea floribunda* leaves extract

The leaves of *Litsea floribunda* have been collected from the forest of Nilgiri hills. The collected leaves 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 acetone at room temperature for 3 days, and filtered. The combined filtrate were concentrated to dryness by rotary evaporation at 50°C and kept in a freezer. In preparing test concentrations of *Litsea floribunda* leaves extract were volumetrically diluted in acetone.

### 2.2 Mosquito culture

Mosquito larvae/eggs of *Culex quinquefasciatus* have been collected in an around Ooty. The mosquito colonies were maintained at 27 ± 2 °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/moulted larvae were used for the bioassay tests. The required quantity of different concentrations of *Litsea floribunda* leaves extract 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. Larvae food consisted of 1g of finely ground dog biscuits per day per trough. Dried coconut midribs were placed over water as the substratum for pupation. The plastic trough containing 200 ml of rearing water with acetone served as the control. Dead larvae and pupae were 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

*C. quinquefasciatus* fresh adults were exposed to filter paper treated with different concentrations of *Litsea floribunda* leaves extracts. The paper was kept inside the beaker. Muslin cloth covering the beaker was also treated. Control insects were exposed only to distilled water with acetone treated paper and muslin cloth. Mortality count was taken after 24h (Sharma *et al.*, 1992).

## 2.5 Ovipositional Assay

Different quantities of *Litsea floribunda* leaves extracts 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 *C. quinquefasciatus*. The gravid females were given a choice between treated and control jars. During the tests, the groups of females were kept separate for 48h in cages measuring 25x 25x30cm. After the eggs were counted the oviposition activity index (OAI) was calculated using the formula:

$$OAI = (N_c - N_t) / (N_c + N_t) \times 100$$

Where  $N_c$  is the number of eggs in the control

$N_t$  is the number of eggs in the treatment

## 2.6 Ovicidal assay

*C. 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 acetone was used (Sharma *et al.*, 1992).

## 2.7 Repellency activity

Different concentrations of *Litsea floribunda* leaves extract were mixed thoroughly with 10ml of goat blood in glass plates. The untreated blood served as the control. Adult females were released into each cage. The number of females landing on the treated blood and untreated blood were recorded. The repellent index of the *Litsea floribunda* leaves extracts were calculated as previously described (Murugan and Jeyabalan, 1999).

## 2.8 Biting deterrence activity

The percentage protection in relation to dose method was used (WHO, 1996). Blood starved female *C. quinquefasciatus* (100 nos), 3-4 days old, was kept in a net cage (45x30x45 cm<sup>2</sup>). The arm of the test person was cleaned with isopropanol. After air drying the arm, a 25 cm<sup>2</sup> area of the dorsal side of the skin was exposed, the remaining portion was covered by rubber gloves. The *Litsea floribunda* leaves extracts were dissolved in acetone, distilled water with acetone served as control. Different concentrations of the *Litsea floribunda* leaves extracts were applied. The control and treated arms were introduced simultaneously into the cage. The numbers of bites were counted over 5 min. from 6 pm to 6 am. The experiment was conducted five times. The percentage protection was calculated by using formula

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

## 2.9 Statistical analysis

All data was subject to analysis of variance and the treatment mean was separated by Duncan's Multiple Range Test (Duncan, 1955).

### 3. Results

Mosquito larvicidal activity of the leaf extracts of *Litsea floribunda* was investigated against *C. quinquefasciatus* mosquito larvae. The larvicidal assays of leaf extracts of *Litsea floribunda* displayed significantly against *C. quinquefasciatus* larvae (75-100%). Those include the Soxhlet extracts in acetone. Complete mosquito larvicidal activities were obtained from the leaf extracts sonicated in water and Soxhlet extracted in acetone. These extracts displayed 100% larvicidal activity at 2% with 1<sup>st</sup> instar larvae of *C. quinquefasciatus*. The percentage mortality of larvae to various concentrations of *Litsea floribunda* was dose dependent, with higher concentration cause in higher larval mortality (Table 1). The extracts among the three concentrations tested, the highest dosage was the most effective against larvae. In 48 h, treated larvae showed nervous movement, followed by falling to the bottom of test cups, subsequently convulsions and death. The present results showed that, mortality percentage in larvae of mosquito species increased by increasing concentration and time of exposure. No larval mortality was observed in untreated control.

The crude acetone leaf extracts of the plant tested against the immature stages of *C. quinquefasciatus* exhibited varied pupal mortality between extracts concentration tested. The acetone extract was found to exhibit the highest pupicidal activity in *Litsea floribunda*. Overall assessment indicated the acetone extract of *Litsea floribunda* to exhibit the highest ovicidal activity. The results indicated in Table 1 existence a relationship between the concentrations used and the percent of mortality. This relationship differed according to the type of solvent used in the extraction. It was found that when using the solvent of acetone, the percent of mortality of first instar larvae was 100% at 2% concentration and decreased to 87% at the concentration of 1% but when the concentration increased to 2% we note an increase in the percent of mortality to 100%.

**Table 1. Effect of acetone extracts of *Litsea floribunda* on the larval Mortality of *C. quinquefasciatus***

Treatment	I instar	II instar	III instar	IV instar
control	00 <sup>d</sup>	00 <sup>d</sup>	00 <sup>d</sup>	00 <sup>d</sup>
<i>Litsea floribunda</i> (%)				
0.5	55 <sup>c</sup>	48 <sup>c</sup>	45 <sup>c</sup>	38 <sup>c</sup>
1.0	87 <sup>b</sup>	75 <sup>b</sup>	70 <sup>b</sup>	68 <sup>b</sup>
2.0	100 <sup>a</sup>	95 <sup>a</sup>	92 <sup>a</sup>	90 <sup>a</sup>

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

The pupicidal effect of the tested plant against *C. quinquefasciatus* in Table 2. Based on mortality values, *Litsea floribunda* proved as highly toxic to mosquito pupae and this response was time dependent. The exposure also delayed pupation five folds longer and the few adults that emerged showed grossly impaired flight ability compared to the non-exposed controls. The results of the adulticidal activity of acetone leaf extract of *Litsea floribunda* against the adult of important vectors mosquitoes, *Culex quinquefasciatus* are presented in Table 2. The vectors tested the highest adulticidal activity was observed acetone than control. The vectors tested the highest adulticidal activity was observed acetone extract of *Litsea floribunda* at 2% concentration. At higher concentrations, the adult showed restless movement for sometimes with abnormal wagging and died. The plant crude extract mortality increased proportionally with dosage. Among the tested plant extract, the highest adulticidal effect was found to be in acetone extract against *C. quinquefasciatus*. The control was tested without solvent no mortality (Table 2).

**Table 2. Effect of acetone extracts of *Litsea floribunda* on the pupa and adult of *C. quinquefasciatus***

Treatment	Pupa mortality (%)	Adult mortality (%)	Adult emergence (%)
control	00 <sup>d</sup>	00 <sup>d</sup>	100 <sup>a</sup>
<i>Litsea floribunda</i> (%)			
0.5	38 <sup>c</sup>	32 <sup>c</sup>	80 <sup>b</sup>
1.0	62 <sup>b</sup>	58 <sup>b</sup>	68 <sup>c</sup>
2.0	78 <sup>a</sup>	72 <sup>a</sup>	52 <sup>d</sup>

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

Increasing trends of mortality were found against larval, pupal and adult stages of *Culex quinquefasciatus* with raising concentrations (1-2%) of leaf extracts. A significant mortality was noticed in all applied solvent systems however, best mortality was observed in acetone extracts than control. Table 2 shows the adult emergence inhibition activity of *Litsea floribunda* acetone extract against *Culex quinquefasciatus*. More emergence of adults occurred from the control containers. The emergence of adults was restricted in those containers that were containing *Litsea floribunda* extract solutions. The adult's emergence from the containers containing the highest extract concentration at 2% was 52%. The extracts showed a high activity with emergence of mosquito paralysis. However, no such effect was observed in untreated insects. On contrary at of almost all the contraction of plant extracts decreased adult periods. The percentages of adult emergence were greatly reduced especially at the highest concentrations. Adult emergence was completely stopped at 2% of *Litsea floribunda* against *Culex quinquefasciatus*.

**Table 3. Developmental duration of *C. quinquefasciatus* after the treatment of acetone extract of *Litsea floribunda***

Trentment	Larval duration (Days)			
	I instar	II instar	III instar	VI instar
Control	1.8 <sup>d</sup>	2.2 <sup>d</sup>	4.2 <sup>d</sup>	4.6 <sup>d</sup>
<i>Litsea floribunda</i> (%)				
0.5	2.6 <sup>c</sup>	3.4 <sup>c</sup>	4.6 <sup>c</sup>	5.2 <sup>c</sup>
1.0	3.8 <sup>b</sup>	4.2 <sup>b</sup>	5.5 <sup>b</sup>	6.8 <sup>b</sup>
2.0	5.7 <sup>a</sup>	6.2 <sup>a</sup>	6.8 <sup>a</sup>	7.2 <sup>a</sup>

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

The larvae took more time to develop to pupae in all the concentration compared to the control (Table 3). At maximum concentration of 2% with all the *Litsea floribunda* gave prolonged the larval duration by all the instars compared with the control, the total development period was increased concentration of treatment. Does response relationship was determined for plant extract applied to *Culex quinquefasciatus*. The duration of larval instars and the developmental time were prolonged. In the present study, application *Litsea floribunda* greatly affected the growth of *Culex quinquefasciatus*.

**Table 4. Pupal and adult duration of *C. quinquefasciatus* after the treatment of acetone extract of *Litsea floribunda***

Treatment	Total pupal duration (day)	Total adult duration (day)
control	3.5 <sup>d</sup>	7.1 <sup>a</sup>
<i>Litsea floribunda</i> (%)		
0.5(%)	5.2 <sup>c</sup>	5.2 <sup>b</sup>
1.0(%)	6.8 <sup>b</sup>	4.3 <sup>c</sup>
2.0(%)	8.6 <sup>a</sup>	3.4 <sup>d</sup>

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

During development metamorphosis, time taken for total larval and pupal development periods (in day), and emergence inhibition were recorded. Results revealed that treated individuals took prolonger larval and pupal period when compared to control. Larval and pupal duration significantly increased in treated individuals and total developmental period (larval and pupal development) in *Culex quinquefasciatus*. The data also revealed gradual increase in pupal duration decrease in adult longevity (Table 4).

**Table 5. Effect of acetone extracts of *Litsea floribunda* on adult repellency and oviposition deterreny of *Culex quinquefasciatus***

S.No	Treatment	Concentration (%)	Adult repellency (%)	Ovipositional deterreny (%)
1	Control		00 <sup>d</sup>	00 <sup>d</sup>
2	<i>Litsea floribunda</i>	0.5	43 <sup>c</sup>	51 <sup>c</sup>
		1.0	64 <sup>b</sup>	74 <sup>b</sup>
		2.0	81 <sup>a</sup>	94 <sup>a</sup>

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

Results of laboratory testing for plant extract of *Litsea floribunda* was screened for adult repellency (%) and ovipositional deterreny (%). It was very interesting to note that in comparison with all our treated groups, acetone extract showed the highest adult repellency (%) and ovipositional deterreny (%). In respect to adult repellency (%), *Litsea floribunda* recorded the least activity among all the concentration (43%, 64% and 81%). Similar observation in respect to concentration gradient was observed for ovipositional deterreny (%) for acetone extract (Table 5). The skin repellent activity of acetone extract of against blood starved adult female of *C. quinquefasciatus* are given in Table 5. The acetone extract had strong repellent action against mosquitoes as it provided 100% protection in the hihger concentrations (2%) against *Culex quinquefasciatus* for 24 hours the mosquito species. It clearly shows that repellent activity was dose dependent. From the results we concluded that the leaf extract of *Litsea floribunda* an excellent potential for controlling *C. quinquefasciatus*.

Table 6 shows the range of number of eggs laid by the gravid female *C. quinquefasciatus* adults is control containers and in extract solution containers. In control containers, the number of eggs was in the range of 240 eggs but in containers with lowest concentration at 0.5% eggs was 185. The highest concentration (2%) of *Litsea floribunda* extract greatly affected the gravid female *C. quinquefasciatus* mosquito.

**Table 6. Effect of acetone extracts of *Litsea floribunda* on fecundity and egg hatchability of *C. quinquefasciatus***

Treatment	Fecundity (no of egg)	Egg hatchability (%)
control	240 <sup>a</sup>	100 <sup>a</sup>
<i>Litsea floribunda</i> (%)		
0.5	185 <sup>b</sup>	76 <sup>b</sup>
1.0	140 <sup>c</sup>	58 <sup>c</sup>
2.0	82 <sup>d</sup>	32 <sup>d</sup>

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

**Table 7. Effect of acetone extracts of *Litsea floribunda* on larval-pupal intermediate of *C. quinquefasciatus***

Treatment	Larval-pupal intermediate (%)
Control	00 <sup>d</sup>
<i>Litsea floribunda</i> (%)	
0.5	25 <sup>c</sup>
1.0	42 <sup>b</sup>
2.0	78 <sup>a</sup>

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

**Table 8. Effect of acetone extracts of *Litsea floribunda* on biting deterreny of *C. quinquefasciatus***

Treatment	Biting deterreny (%)
Control	00 <sup>d</sup>
<i>Litsea floribunda</i> (%)	
0.5	18 <sup>c</sup>
1.0	52 <sup>b</sup>
2.0	81 <sup>a</sup>

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

Effects of plant extracts with experimental solvents against *Culex quinquefasciatus* was examined for biting deterreny (Table 8). In this observation, all the crude extracts of solvents gave protection against mosquito bites without any allergic reaction to the test persons and also the biting deterreny activity is dependent on the concentration of the plant extracts. When analyzed the effects of acetone extracts against *Culex quinquefasciatus* on biting deterreny, against 0.5%, 1% and 2% concentrations, it was observed that *Litsea floribunda* recorded that the least biting deterreny in all concentration (0.5%, 1% and 2%) with values of 18%, 52% and 81% respectively. When compared with methanol extracts biting deterreny was recorded highest comparable with acetone and petroleum ether extracts.

## 4. Discussion

Mosquito-borne diseases, such as malaria, filariasis, dengue, yellow fever, and Japanese encephalitis, contribute significantly to disease burden, death, poverty, and social debility in tropical countries such as Ghana (Jang *et al.*, 2002). Natural products are generally preferred in vector control measures due to their less deleterious effect on non-target organisms and their innate biodegradability. With respect to resistance developed by the mosquito larvae and pupae against chemical insecticides, it is worthwhile to identify new active compounds from natural products against mosquitoes. The outcome of the present research obviously shows that *Litsea floribunda*. *Litsea floribunda* have astonished mosquito properties against *Culex quinquefasciatus* larvae and pupae. The direct and indirect contributions of such effects to treatment efficacy through reduced larval and pupal feeding and fitness need to be properly understood in order to improve the use of botanical insecticides for of *A. gambiae* (Subramaniam *et al.*, 2012). These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future (Wandscheer *et al.*, 2004).

The aquatic immature larval stage is recognized as the most vulnerable and best control strategy to effectively reduce mosquito population densities during infestations (Goddard, 2000). This study showed that botanical extract can be suitable alternatives to the use of synthetic insecticide in the control of insect vectors of economic importance. The plant assessed had proven high insecticide ability against mosquito and this was in agreement with Ileke *et al.* (2014), who reported the insecticidal potential of botanicals like *Anacardium occidentale*, *Afromomum melegueta*, *Garcinia kola*, *Citrus sinensis*, *Clerodendrum capitatum* and *Bridelia machranth* plants against malarial vector mosquito, *Anopheles gambiae*.

In the present study the *Listsea floribunda* plant species were identified for mosquito control in Nilgiris. The presence of several plant species used for mosquito control by local people is a good indication of the deep-rooted culture of local plants in the study area. In the present result is similar to other studies of two plants conducted in Ethiopia (Belkew *et al.*, 2012). The current results show that local communities had indigenous knowledge and give emphasis to using local plants to repel mosquitoes. Some of the repellent plants have been reported in other parts of Ethiopia (Karunamoorthi *et al.*, 2009).



Plants contain secondary metabolites and the effectiveness of these secondary metabolites such as alkaloids, isoflavonoids, saponine and steroids has potential mosquito larvicides and pupicides (Venkandachalam and Jebasan 2001). These essential oils could also affect the swimming ability of the mosquito larvae and pupae; thereby hinder their swimming to the surface for oxygen which could lead to their death. The strong choky odour of the plants may have also disrupted respiratory activity of the insect (Ileke *et al.*, 2013), blocking the spiracles thereby hinder the larvae breathe (Ileke *et al.*, 2018). This result into asphyxiation and death of the larvae (Ileke *et al.*, 2015).

The test plants were found to have potential larvicidal activities against all the instar of larvae of *Culex quinquefasciatus* at the test concentration. The toxicity difference and extraction solvent among test plants suggested that different plant have different phytochemicals, which can be extracted by different solvents. High larval mortality was caused by acetone leaf extract of *Litsea floribunda*. This finding is similar to the report of Okumu *et al.* (2009). Where acetone leaf extract of *Litsea floribunda* was found to cause 100% mortality against first instar larvae of *C. quinquefasciatus* at 2% concentration. This could be due to the presence of excess bioactive secondary metabolites (Kortbeek *et al.*, 2019).

Similarly, acetone extract of *Ocimum lamifolium* oil was found to have high larvicidal activity against *An. arabiensis* (Fekadu *et al.*, 2009). Various factors might be responsible for the larvicidal activity, but the difference in larvicidal activities in the current finding could be due to locality of the plants, and solvents. In the present study the acetone leaf extract of *Litsea floribunda* causes adult mortality 72%, which could be due to the presence of bioactive secondary metabolites. This finding is in line with the earlier finding of Kamaraj *et al.* (2010) who reported adulticidal efficacy of methanol against *Culex gelidus* Teobald.

Sosan *et al.* (2001) reported larvicidal activities of essential oils of *Ocimum gratissimum*, *Cymbopogon citratus* and *Ageratum conyzoides* against *Ae. aegypti* and achieved 100% mortality at 120, 200 and 300 ppm concentrations respectively. Similarly, it was reported that the essential oil of *Ipomoea cairica* Linn possesses remarkable larvicidal properties as it could produce 100% mortality in the larvae of *Cx. tritaeniorhynchus*, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* mosquitoes at concentrations ranging from 100 to 170 ppm (Thomas *et al.*, 2004). Dwivedi and Kawasara (2003) found acetone extract of *Lantana camara* to be most effective against *Cx. quinquefasciatus* larvae at the dose of 1 ml/100 ml. Latha *et al.* (1999) reported *Piper longum* and *Zingiber wightianum* extracts at 80 mg/l causing complete mortality in *Cx. quinquefasciatus* and 60 mg/l for *Cx. sitiens*.

Larvicidal activities of the plant extracts vary according to the plant species, the parts of the plant, the geographical location where the plants were grown and the application method. Plant could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these plant extracts for development of eco-friendly chemicals for control of insect vectors.

Higher concentrations of the leaf extract were needed to achieve higher mortality on pupae than on larvae. This indicates that the larvae is more susceptible to these plants than pupae and this could be attributed to the active feeding stage of the larvae since pupae stages of the insect are not feeding (Ileke *et al.*, 2015).

Although studies have shown the effectiveness of herbal repellent products, when applied to the skin, the protection provided usually dissipates rapidly (Li *et al.*, 2013). In the case of essential oils, this fact is related to high volatility, a property that may be enhanced with the development of formulations that could be able to keep the principles active for an extended period. Considering the high volatility of EO, repellent performance is heavily dependent on the product's composition and capacity to prolong the duration of action.

According to a study by Choocote *et al.* (2007) a compound combined with essential oil that was demonstrated to increase repellency time was vanillin. Additionally, the repellency time of pure oil was shorter when compared with the mixture of 10% vanillin. Kazembe and Nkomo (2012) analyzed the formulation of crème with crude extract of leaves *Ocimum gratissimum* and *Lantana camara* L. which presented repellent activity against *Aedes aegypti* L., without exhibiting adverse reactions in the volunteers. Therefore, fixing additives and the production of repellents combined are possibilities that can increase the effectiveness and economic value of essential oils with repellent activity.

Hill *et al.* (2007) described a standardization of the repellent product based on a biomarker. The substance used as a marker was p-mentan-3,8-diol (PMD), an essential oil classified as monoterpene and that can be extracted from aromatic plants such as eucalyptus (*Corymbia citriodora*) and has proven insect repellent properties similar to DEET (Carroll and Loye, 2006). Despite the findings, there are some difficulties inherent to the development of the herbal product, due to the complexity of the plant's constituents. The raw material and the variability in the quality of the products obtained from the plant species. These characteristics are related to the factors and conditions of the place of cultivation, the procedure of collection, handling, and processing of the vegetable raw material. Thereby, plant products will show variations, further increasing the requirement for standardization to guarantee efficacy, safety, and quality (Liu *et al.*, 2011).

This study clearly demonstrates the presence of many medicinal plants as insecticidal, repellent and larvicidal agents. Therefore, this information indicates the need of research to be carried out on the bioactive compounds present in the particular plants which have a potential against insects as a solution of the deleterious effects of synthetic insecticides, including lack of selectivity, impact on the environment and the emergence and spread of pest resistance. From these medicinal plants there will be a promising role in the development of future commercial insecticidal agents in terms of larvicidal, antifeedant, repellent, oviposition deterrent and growth regulatory used as a preventive medicine point of view.



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