JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

BIOLOGICAL ACTIVITIES OF *CARDIOSPERMUM HALICACABUM* LEAF EXTRACT AGAINST FILARIAL VECTOR, *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

Roja, H. R. and Jeyabalan, D.

Department of Zoology, Government Arts College, Udhagamandalam- 643 002
The Nilgiris, Tamil Nadu, India

Abstract:

Mosquitoes (Diptera: Culicidae) species are vector responsible for the transmission of several infections with medicinal and veterinary importance including filariasis, malaria, arboviruses. *Culex quinquefasciatus* is the major vector of the bancroftian filarial parasite which causes human lymphatic filariasis and St. louis encephalitis. Using chemical insecticides released into the environment may have adverse biological effects. Therefore, there is a need for ecofriendly insecticides for mosquito control. The currents study was aimed investigated larvicidal, pupicidal, adulticidal activities of ethanol extracts of *Cardiospermum halicacabum*. It was more effective against the *Culex quinquefasciatus* on larvicidal, pupicidal, adulticidal, repellent, biting deterrent, reproductive, growth regulatory and deformities after the treatment of *Cardiospermum halicacabum* leaf extracts.

Key words
Cardiospermum halicacabum, *Culex quinquefasciatus*, larvicidal, pupicidal, adulticidal, repellent.

1. INTRODUCTION

Mosquitoes belong to a high-risk category of indoor pests, with established identity as vectors for the transmission in human and animal diseases. They represent a diverse family of insects called Culicidae, with 3 subfamilies like *Toxorhynchitinae, Anophelinae* and *Culicinae* comprising more than 3400 species (Reiter, 2001). Mosquitoes enjoy worldwide distribution marking their presence in tropical and temperate regions, including Arctic zone. But they are not recorded from Antarctic zone. They have been reported from high altitudes up to 6000m above sea level on the mountains and 1250m below the sea level in the mines and caves (Lane *et al*., 1993). Latitudinal distribution of mosquitoes has recorded differential patterns for various species. Highest species diversity of mosquitoes has been reported from the Southeast Asia and the Neotropics (Gaston, 1994). Mosquitoes have attained a notorious status, through the numbers and types of diseases transmitted by them and the extent of disaster they cause to public health. Despite the technological developments in the healthcare sector, mosquito-borne diseases pose serious challenges.

Mosquitoes are serious human disease causing insects which transmit many dreadful diseases and therefore they are considered as 'public enemy number one' (Reegan *et al*., 2015). Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally and 40,000,000 of the people of Indian population. They act as a vector for most life threatening diseases like malaria, yellow feaver, dengue fever, chikungunya fever, filariasis, encephalitis, west nile virus infection etc., in almost all tropical and subtropical countries and many other parts of the world.

Mosquitoes are the important single group of insects in terms of public health importance and causing millions of death every year (Das *et al*., 2004). These diseases not only cause high levels of morbidity and mortality, but also inflict great economic loss and social disruption on developing countries such as India, China etc., India alone contributes around 40% of global filariasis burden and the estimated annual economic loss is about 720 crore (Kamaraj *et al*., 2011)

Culex quinquefasciatus serves as the main vector of Bancroftian filariasis, both in tropical and subtropical areas (Abbey *et al*., 1990; CDC, 2016). More than 120 million people suffered from filariasis, of which 40 million developed disabilities due to the disease. *Culex quinquefasciatus* more commonly called the southern house mosquito, is the principal vector of lymphatic filariasis caused by *Wuchereria bancrofti* and a potential vector of Dirofilariaimmitis (Bhattacharya *et al*., 2016). It is one of the most widespread mosquitoes in the world. Lymphatic filariasis is probably the fastest spreading insect-bornes disease of humans in the tropics. The disease has a focal distribution, and it is estimated that currently over 2.5 million people are at risk of acquiring filariasis (Lotfy, 2014). Both diseases not only

cause mortality and morbidity among humans but also cause social, cultural, environmental and economic loss to the society (Ghosh *et al*., 2013).

Culex quinquefasciatus Say (Diptera: Culicidae) is one of the major domestic pests in urban areas and carry *Wuchereria bancrofti*, the lymphatic filarial worm, and many arboviruses. Lymphatic filariasis is the second leading cause of permanent and long-term disability in the world. In India, filariasis is endemic in 250 districts from 17 states and 6 union territories, also about 553 million people at risk of infection (Raju *et al*., 2010). It is a brown-coloured medium-sized mosquito found in tropical and sub-tropical regions of the world has been established as the vector of *Wuchereria bancrafti*, avian malaria and arboviruses including *St. Louis encephalitis* virus, western equine encephalitis virus, West Nile virus, various protozoans etc. *Lymphatic filariasis* which is caused by the parasitic infection (e.g. *Wuchereria bancrafti*) and transmitted by *C. quinquefasciatus* can affect the lymphatic system of human beings (Arunachalam *et al*., 2014). About 1.10 billion people are threatened by this disease in 58 countries worldwide. In India, 19 million people suffer from filarial disease manifestations (Kovendan and Murugan, 2011). The adaptive fitness, host specificity, high reproductive capacity etc. has made *C. quinquefasciatus* smart vector (Bhattacharya and Basu, 2016).

The termination of the disease transmission cycle may be done by elimination of mosquito breeding places as well as eradication of larvae and adult mosquitoes (Das *et al*., 2007). According to its application, there are various forms of domestic insecticide, one of which is the electric liquid vaporizer (Elango *et al*., 2010). Using an electrical current, the electric liquid vaporizer is able to produce heat that will lead to production of insecticide vapor, causing mosquito death (Elango *et al*., 2009). Prallethrin is a synthetic pyrethroid that has rapid knockdown activity against domestic pest insects (Elango *et al*., 2010).

Mosquito control programs are crucial for the control of these diseases. The multiple devices and approaches used to fight against these illnesses predominantly depend on disrupting transmission pathways by targeting mosquito larvae and adults. For example, organophosphates, insect growth regulators, or microbial agents were usually applied to stagnant water which can serve as a breeding ground of mosquitoes. For indoor settings, residual spraying and insecticide-treated mosquito nets are usually applied to kill adult mosquitoes using pyrethroids (Yakob *et al*., 2011). However, the over use of chemical components to control mosquitoes is detrimental to the environment and human health. Furthermore, insecticide resistance has rapidly developed in a number of mosquito species (Naqqash *et al*., 2016). Therefore, there is an urgent need to develop an eco-friendly vector control strategy. Recently, much attention has been focused on essential oils (EOs) as alternative agents due to their low mammalian toxicity and bio-degradability in the environment. Moreover, the multiple bioactive constituents of EOs with intricate action modes can substantially reduce the opportunity for insecticide resistance in mosquitoes. Approximately, 17,500 aromatic plant species have been reported to contain EOs, in particular, *Asteraceae*, *Lauraceae*, *Lamiaceae* and *Myrtaceae* are major plant families from which EOs are extracted (Regnault-Roger *et al*., 2012). EOs has exhibited a variety of industrial applications, such as perfumery, cosmetics, detergents, pharmacology, chemistry, and food production (Pavela, 2015).

Recent mosquito-control research has been focusing on the interruption of disease transmission either by killing, preventing the disease-vectors, mosquitoes, from biting humans or by killing the larvae at their breeding sites. The wide use of conventional chemical insecticides, such as malathion and DDT, against adult mosquitoes have shown promising results in combating the spread of mosquitoes. However, several mosquito strains developed resistance to those chemical pesticides (Brown, 1986) in addition to their apparent side-effects as they have found to be toxic (such as leaving toxic residues on treated crops) and have adverse effects on the environment (by contaminating air, water, and soil) humans and animals (Lee, 2000).

Plant products have been used traditionally to repel and kill mosquitoes in many parts of the world. Thousands of plants have been tested as potential sources of insect repellents. The use of medicinal plants is an ancient practice used by populations to cure various diseases. This practice is expanding all over the world. Medicinal plants constitute an important and readily available resource found in popular markets, backyards, and areas of native vegetation. Plants are the store house of various phytochemicals such as terpenoides, alkaloids, steroids, resins, saponins and oils which act on insect's body in different ways (Venkatachalam and Jebanesan, 2001). Phytochemical based insecticides are considered to be more eco-friendly, biodegradable and safer than synthetic insecticides. Botanicals with mosquitocidal properties such as general toxicant, repellents, growth and reproductive inhibitors and oviposition- deterrents have been projected as potent alternative natural insecticides in future mosquito control programmes (Sukumar *et al*., 1991).

Various plant species have been exploited throughout the world to control the mosquito populations (Muthukrishnan and Puspalatha, 2001). Secondary metabolites obtained from the indigenous plants with proven mosquito control potential can be used as an alternative to synthetic insecticides under the integrated vector control program. The chemicals derived from plants have been projected as weapons in future mosqutio control program as they are shown to function as insect growth regulators, repellents, and ovipositional attractants, having deterrent activities observed by different researchers (Murugan *et al* .,1996; Venkatachalam and Jebanesan, 2001) studied the potential of natural products as larvicides against the main African -malaria vector, *An. gambiae.*

Plant phytochemicals have more specific effects and could be usefully integrated with other control measures to design comprehensive, appropriate and effective management protocols with less collateral harm to the environment and effective management protocols with less collateral harm to the environment and non-target species (Silva *et al.*, 2010). Phytochemicals have advantageous due to their eco-safety, target-specificity, non-development of resistance, reduced number of application, higher acceptability and suitability for rural areas. Botanicals can be used as an alternative to synthetic insecticides or long with other insecticides under integrated vector control programs. The plant product of phytochemical, which is used as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites. Phytochemicals obtained from the whole plant or specific part of the plant by the extraction with different types of solvent such as aqueous, ethanol, chloroform, benzene, acetone, petroleum ether, etc., depending on the polarity of the phytochemicals. Some phytochemicals act as toxicant (insecticide) both against adult as well as larval stages of mosquitoes, while others interfere with growth and growth inhibitor or with reproduction or produce an olfactory stimulus, thus acting as repellent or attractant

(Markouk *et al.,* 2001).

Govindarajan (2011) investigated the larvicidal and ovicidal activities of benzene, haxane, ethyl extract of *Caesalpinia pulcherrima* against *Culex quinquefasciatus, Aedes aegypi* and *Anopheles stephensi* showed that the crude extract for controlling mosquitoes. Larvicidal activity of *Moringa oleifer* a was demonstrated by Govindarajan *et al.* (2011) which exhibited in the first to fourth instar larvae of the *Anopheles stephensi* and the LC_{50} and Lc_{90} values were 57.79 ppm and 125.93 ppm for first instar, 63.90 ppm and 133.07 ppm for second instar, 72.45ppm and 139.82 ppm for third instar,78.93ppm for fourth instar respectively. Thus, does decides the mortality and developmental stages is also taken into account, in determining the toxicity (Prabhu *et al.,* 2011).

Dhar *et al.* (1996) reported that effect of neem oil volatiles on gonadotrophic cycle and inhibition of oviposition in *An. stephensi* and *An. culicifacies*. Jeyabalan *et al.* (2003) tested the effect of methanol extracts of Pelargonium citrose leaf for their biological, larvicidal, pupicidal, aduliticidal, antiovipositional activity, repellency and biting activity of *An. sephensi*. Murugan and Jeyabalan (1999) evaluated the larval toxicity of botanical pesticides such as Neem *(Azadirachta indica)*, Pongam (*Pongamia pinnata .L*), Thumbai (*Leucas aspera*) along with *Baciluss phaericus* (*Bs*) on the filarial vector, *Culex quinquefasciatus.*

Mosquito larvicidal, ovicidal and pupicidal activities of *Annonoa reticulate* (Balu Selvakumar *et al.,* 2015) and *Cuscutare flexa* (Sweta *et al.,* 2015), *Parthenium hysterophorous* (Nisar Ahmad *et al.,* 2011) against *Culex pipiens* and *C. quinquefasciatus* elicited that ethanolic extract proven to exhibit significant effect that benzene, petroleum ether and acetone. As the concentration increased, the ovicidal, larvicidal and pupicidal activities increased on the other hand fecundity rate decreased (Shyamapada Mandal, 2011). Similarly Vincent *et al.* (2015) worked with the American weed, *Parthenium hysterophorus* to control the malarial combat in Africa.

Daniel *et al.* (1995) reported that there was a prolonged larval and pupal period on *Culex quinquefasciatus* when treated with the methanolic extracts of *Acalypha indica*. Deshmuk *et al.* (1987) showed that petroleum ether extract of *Acorus calamus* and *Azadirachta indica* showed excellent insect growth regulatory effect against *Culex pipiens fatigans*. Sharook *et al.* (1991) found that acetone extracts from *Melia volkensii* and *Melia azedarach* seeds exhibited growth inhibitory activity against *Culex pipiens* larvae, mostly at 1st and 2nd instar stages, on the other hand 3rd instar and 4th instar were less susceptible to the extracts. Supavaran *et al.* (1974) reported ethanolic extract of whole plant of *Anethum graveolens* toxic to fourth instar larvae of *Aedes aegypti* and high inhibition of pupal development. Mohtar *et al.* (1999) reported ethanolic aqueous extract of *Nerium indicum* leaves on different larval stages of *Aedes aegypti* and an elongation of the pre-imago period on the treated larvae. Many plants produce secondary components that have insect growth inhibitory activity. Indeed, many plant extracts were analyzed to kill larvae of different species of mosquitoes (Girdhar *et al.,* 1988). The phytochemicals derived from plant sources can act as larvicides, insect growth regulators and repellents (Alakarmalai Jeyasankar *et al.,* 2012). Arivoli *et al.* (2012) reported the larvicidal activity first instar larvae of *Anophelus stephensi*.

The pesticides of plant origin are efficient, biodegradable as well as a suitable alternative for mosquito control. The phytochemicals derived from plant resources can act as larvicides, insect growth regulators, repellents and ovipositional atteractants, having deterrent activities (Babu and Murugan, 1998; Venkatachalam and Jebanesan, 2001). Shaalan *et al.* (2005) has reviewed on different mosquito larvicidal plant species with growth retarding, reproduction inhibiting, ovicides, synergistic, additive and antagonistic activities of botanical mature. Raven *et al.* (1999); Kovendan and Murugan (2011); Ghosh *et al.*(2012) worked on different phytochemicals with various solvent extracts and found that ethanolic extracts were effective against larval form than hexane and petroleum ether. Carabolla (2000); Seyom *et al.* (2002); Thangam and Kathiresan (1991) worked with botanical against *Culex quinquefasciatus* against larvicidal activity with extracts of various solvents and proved the efficacy of ethanolic extracts to be the maximum at larval stages. Specifies target -insects and are eco-friendly (Sukumar *et al.,* 1991). Therefore, the present study was carried out to evaluate the mosquitocidal property of *Cardiospermum halicacabum* leaf extracts against the filarial vector, *C. quinquefasciatus*.

2. MATERIALS AND METHODS

Collection and preparation of plant extracts

Healthy leaves of *Cardiospermum halicacabum* was collected from Gudalur area, The Nilgiris of Tamilnadu, India. The plant was 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 solvent (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 were volumetrically diluted in solvent.

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).

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. Larvae 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).

LC_{50} and LC_{90} values and their 95% confidence limits were estimated for larval mortality by fitting a probit regression model to the observed relationship between percentage mortality of larvae and logarithmic concentration of the substance. Separate probit models were fitted for each extract (Finney, 1971)

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.

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 solvents treated paper and muslin cloth. Mortality count was taken after 24h (Sharma *et al*., 1992).

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

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

Repellency activity

A different concentration of plant extract was 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 extract was calculated as described by (Murugan and Jeyabalan, 1999).

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 extract 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:

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

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

Present study was analyzed locally grown *Cardiospermum halicacabum* are collected and the ethanolicggggg extracts of their leaves were tested for larvicidal, pupicidal, adulticidal, larval duration, pupal duration, adult duration, reproductive activity, repellency and

biting deterrence of *Culex quinquefasciatus*. The assay of investigated plant species (*Cardiospermum halicacabum*) were carried out using different concentration in ethanol extracts against *Culex quinquefasciatus*. The plant was more effective at high concentrations, the toxic effect however increased with increase in the concentrations of the extract. A moderate effect of plant extracts were observed at lower concentration but exhibited higher activity as the concentration increased.

Table 1. Effect of *Cardiospermum halicacabum* on the larval mortality of *Culex quinquefasciatus*

S. No	Treatment	Concentration(%)	1st Instar	2nd Instar	3rd Instar	4th Instar
1.	Control	00	00^{d}	00^{d}	00^{d}	00^{d}
2.	*Cardiospermum halicacabum*	0.5	72^{c}	69^{c}	57^{c}	49^{c}
		1	82^{b}	78^{b}	70^{b}	65^{b}
		2	100^{a}	96^{a}	89^{a}	81^{a}

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

Table 1 indicates the leaf extract of *Cardiospermum halicacabum* with ethanol was tested for larvicidal activity against *Culex quinquefasciatus* at variable and increasing concentration of 0.5%, 1%, and 2% and the mortality were recorded for each instar stages. It has been observed that *Cardiospermum halicacabum* registered the mortality values of 100%, 96% , 89% and 81% respectively with 1st instar, 2nd instar, 3rd instar and 4th instar larval stages (Table1) at 4% treatment. It has been observed that with increase in mortality with increase in the concentration of plant extracts. It has also been observed that the 1st and 2nd instar larval forms requires less concentration to bring out the mortality, than the 3rd and 4th instar larval forms, which requires a comparatively high concentration (%) of the extract to bring out the mortality.

Table 2. Effect of *Cardiospermum halicacabum* on the pupae and adult of *Culex quinquefasciatus*

S. No	Treatment	Concentration (%)	Pupal mortality (%)	Adult mortality (%)	Adult emergence (%)
1.	Control	00	00^{d}	00^{d}	100^{a}
2.	*Cardiospermum halicacabum*	0.5	65^{c}	58^{c}	60^{b}
		1	78^{b}	69^{b}	51^{c}
		2	96^{a}	86^{a}	45^{d}

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

The Pupicidal mortality, adulticidal mortality and adult emergence (%) of *Cardiospermum halicacabum* with ethanol extracts is shown in table 2. It was observed that the increase in concentration in test samples was raised with increase in mortality. No mortality was recorded in respective control replicates within the experimental conditions. *Cardiospermum halicacabum* displays a significant pupal mortality at all the concentration. Even at 1% concentration of *Cardiospermum halicacabum* exposes the 78% pupal mortality on the other hand at higher concentration (2%) of *Cardiospermum halicacabum* gives 96% pupal mortality.

The adult mortality (%) of *Culex quinquefasciatus* at various concentration of ethanol extracts of *Cardiospermum halicacabum* is depicted in table 2. The experimented plant showed significant mortality in bioassays with ethanol extracts of *Cardiospermum halicacabum* at 4% concentration. The adult mortality was 58%, 69% and 86% at 0.5%, 1% and 2% respectively after the treatment of *Cardiospermum halicacabum* extracts. It was also noted that when compared with pupal mortality, adult mortality was comparatively lower for all treatments. Where increase in concentration, directly increased the adult mortality, exhibiting a direct relationship with the concentration.

The adult emergence from pupae after permanent exposure to plant with ethanol extracts is represented in Table 2. It is also interesting to note that in the given conditions, the plant exhibited efficient reduction in adult emergence, in comparison with the control. In the study, at higher concentration (2%), *Cardiospermum halicacabum* exhibited 45 % adult emergence, followed 51% adult emergence at 1% and 60% adult emergence at 0.5% of plant extracts. In this case, it was a dose dependent action, where gradual increase in the concentration of the extracts is indirectly proportional to the adult emergence. When compared this result with pupal mortality and adult mortality there exhibited a direct relationship with increase in concentration and analogous result with adult emergence.

Table 3. Effect of *Cardiospermum halicacabum* on the adult repellency and oviposition deterrency of *Culex quinquefasciatus*

S. No	Treatment	Concentration(%)	Adult Repellency (%)	Oviposition Deterrency (%)
1.	Control	00	00^{d}	00^{d}
2.	*Cardiospermum halicacabum*	0.5	45^{c}	57^{c}
		1	62^{b}	72^{b}
		2	84^{a}	97^{a}

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 *Cardiospermum halicacabum* screened for adult repellency (%) and ovipositional deterrency (%). Adult repellency (%) and ovipositional deterrency (%) for ethanol extract is shown in table 3.

In respect to ethanol extract of the plants have the potential of adult repellency activity. *Cardiospermum halicacabum* with 45%, 62% and 84% repellency at 0.5%, 1% and 2% concentration respectively. From this it can be confirmed that the adult repellent activity against *Culex quinquefasciatus* increases with increase in concentration, exhibiting a dose depended response.

Regarding the ovipositional deterrent activity of ethanol extracts of plants against *Culex quinquefasciatus*, it can be inferred that *Cardiospermum halicacabum* exhibits highest ovipositional deterrency with values ranging from 57%, 72% and 97% with respect to the concentration of 0.5%, 1% and 2%. From this it can also be confirmed that the dose plays a major role, which reduced the egg laying when compared the untreated bowl (control).

Table 4. Developmental duration of *Culex quinquefasciatus* after the treatment of*Cardiospermum halicacabum.*

S. No	Treatment	Concentration (%)	1st Instar (days)	2nd Instar (days)	3rd Instar (days)	4th Instar (days)
1.	Control	00	1.6^{c}	2.9^{c}	3.9^{c}	4.5^{c}
2.	*Cardiospermum halicacabum*	0.5	2.6^{c}	3.8^{c}	4.7^{c}	5.8^{c}
		1	4.5^{b}	7.9^{b}	8.8^{b}	9.2^{b}
		2	6.7^{a}	9.6^{a}	10.8^{a}	11.3^{a}

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

After the treatment of mosquito, *Culex quinquefasciatus*, the treated mosquito's larvae were assessed for their larval duration. The larval duration of *Culex quinquefasciatus* after the treatment of plant extracts with ethanol is given in table 4. Comparatively it was observed that the larvae took more time to develop into pupae in all the treatments when compared to the untreated groups - control. At maximum concentration of 2% with the solvent extracts of plants gave prolonged larval duration in all the instars compared with the control, the total developmental period was observed to be increased with increasing concentration of treatments. Dose–response relationship was determined for plants applied to *Culex quinquefasciatus*. Increase in the concentration of the extracts, increase in the developmental duration, which clearly reveals the dose–response relationship. The duration of larval instars and the total developmental time were prolonged. The possible reason could be a harmonic mimic; on the other hand we are not aware of the exact mechanism to reveal this effect. Hence forth, in our present study the application of plant extracts greatly affected the developmental duration at every concentration, which shows promising efficacy and delay in the growth of *Culex quinquefasciatus* which is a satisfying result for further study of these plants.

Table 5. Pupal and adult duration of *Culex quinquefasciatus* after the treatment of*Cardiospermum halicacabum*

S. No	Treatment	Concentration (%)	Total pupal duration (days)	Total adult duration (days)
1.	Control	00	3.2^{c}	61^{a}
2.	*Cardiospermum halicacabum*	0.5	6.5^{c}	43^{b}
		1	8.7^{b}	39^{c}
		2	10.5^{a}	25^{d}

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

The developmental metamorphosis for pupae and adult developmental duration (days) was recorded for the plant with ethanol extracts of *Cardiospermum halicacabum*. The observed results for ethanol are presented in the table 5. Analysis of the adult *Culex quinquefasciatus* developmental metamorphosis against plant extracts of ethanol, time taken for total larval and pupal developmental periods (in days) were significantly inhibited. Analysis of ethanol extracts of plants with respect to pupal and adult duration (days) signifies that it is concentration dependent (Table 5). Increase in the concentration from 0.5%, 1% and 2%, results in decrease total pupal duration which signifies a direct relationship with the concentration, at the same time decrease in total adult duration (days) which shows an inverse relationship with the concentration gradient.

The observed results clarify that treated individuals took prolonged larval and pupal period when compared to control in our test group. Total larval duration increased and total adult duration decreased significantly with increased concentration among our treated individuals of *Culex quinquefasciatus.*

Table 6. Effect of *Cardiospermum halicacabum* on fecundity and egg hatchability of *Culexquinquefasciatus*

S. No	Treatment	Concentration (%)	Fecundity (No. of eggs)	Eggs hatchability (%)
1.	Control	00	205^{a}	100^{a}
2.	*Cardiospermum halicacabum*	0.5	166^{b}	71^{b}
		1	131^{c}	50^{c}
		2	60^{d}	32^{d}

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

Action of ethanol extracts of *Cardiospermum halicacabum* on fecundity (no. of eggs laid) of *Culex quinquefasciatus* were obtained from the general stock of mosquitoes and tested in relation to the different concentrations of ethanol at 0.5%, 1% and 2% respectively (Table 6). The plant had a promising effect on *Culex quinquefasciatus.* When analyzing the number of eggs lay after the treatment of plant extracts with ethanol, it was observed that at all the concentration, the plant exhibited promising efficient effect on the fecundity (Table 6). To the extent of increase in concentration from 0.5%, 1% and 2% of *Cardiospermum halicacabum* extracts recorded 166, 131 and 60 numbers of eggs respectively, in comparison with control of 205 eggs. Thus it can be determined that as the concentration increases, the fecundity (no. of eggs) decreases which is an inversely proportional result and a satisfying result. Thus *Cardiospermum halicacabum* exhibits potent decrease in fecundity in comparison with treated groups with respect to control.

Action of ethanol extracts of *Cardiospermum halicacabum* on hatching of *Culex quinquefasciatus* eggs freshly laid were obtained from the general stock of mosquitoes and tested for their hatching ability (%) in relation to the different concentrations of ethanol at 0.5%, 1% and 2%. It has been noted that as the concentration increases, the egg hatchability decreases. The *Cardiospermum halicacabum* exhibits remarkable result of 71%, 50% and 32% egg hatchability (%) at 0.5%, 1% and 2% respectively, which signifies that the egg hatchability decreases with increase in concentration, exhibiting a dose dependent inverse relationship.

Over all it can be noted that the plant extracts showed promising decrease in fecundity (no. of eggs) and egg hatchability with increase in concentration, demonstrating inverse relationship with dose–concentration effect. In our present study, we enumerated the larval-pupal intermediate (%) of *Culex quinquefasciatus* with various increasing concentration (0.5%, 1% and 2%) of ethanol extracts of *Cardiospermum halicacabum* represented data in Table 7.

Table 7. Effect of *Cardiospermum halicacabum* on Larval-pupal intermediate of *Culex quinquefasciatus*

S. No	Treatment	Concentration (%)	Larval-pupal Intermediate (%)
1.	Control	00	00^{d}
2.	*Cardiospermum halicacabum*	0.5	42^{c}
		1	67^{b}
		2	92^{a}

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

Analysis of larval–pupal intermediate (%) of *Culex quinquefasciatus* with ethanol extracts showed promising determintal effect. The larval-pupal intermediate of *Cardiospermum halicacabum* registered 42%, 67% and 92% intermediates, with increasing concentration of 0.5%, 1% and 4% respectively. Thus increase in concentration of the extracts, increased the retention of *Culex quinquefasciatus* in larval–pupal intermediate stage with ethanol extracts.

The analysis clearly indicates that lower concentration of the plants with solvents effectively produced clear morphological growth disruption in the treated mosquito larvae, pupae and adults compared to controls, showed normal structural features. Several forms of morphological malformations resulted from treatment of larvae, pupae and adults with the extracts. The apodous larvae showed several types of morphological malformations; deformed mouth brushed melanised and sclerotized cuticles. The pupae that survived through larval treatment showed a variety of malformation like complete demelanized pupae with straight abdomen, partly melanised pupa with extended abdomen, dwarf pupa with retarded abdomen, dechitinized pupa with distorted the minalia and pupa with defective genitalia. Among the treated groups, ethanol extracts of *Cardiospermum halicacabum* showed that the least larval- pupal intermediate.

Table 8. Effect of *Cardiospermum halicacabum* on biting deterrency of *Culex quinquefasciatus*

S. No	Treatment	Concentration (%)	Biting deterrency (%)
1.	Control	00	00[d]
2.	*Cardiospermum halicacabum*	0.5	37[c]
		1	71[b]
		2	86[a]

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 deterrency (Table 8). In this observation, the crude extracts of solvents gave protection against mosquito bites without any allergic reaction to the test persons and also the biting deterrency activity is dependent on the concentration of the plant extracts. When analyzed the effects of ethanol extracts against *Culex quinquefasciatus* on biting deterrency, against 0.5%, 1% and 2% concentrations, it was observed that *Cardiospermum halicacabum* recorded that the highest biting deterrency in all concentration (0.5%, 1% and 2%) with values of 37%, 71% and 86%.

4. DISCUSSION

Blood feeding female mosquitoes are responsible for biting irritation and the transients of a large number of diseases, such as malaria, yellow fever, dengue, filariasis, chickenguniya, and encephalitis, which causes serious health troubles to human. And also an obstaclet socio-economic development of developing countries, particularly in the tropical region (Murugan *et al.*, 2007). *Culex* is an important factor of some fundamental diseases, such as West Nile virons, filariasis, Japanese encephalitis, St. Louis encephalitis and avian malaria. *Culex quinquefasciatus* is the cause for transmitting the filarial nematodes, *Wuchereria bancrofti*. More than 1.3 billion people in 72 countries, worldwide threatened by lymphatic filariasis, commonly known as elephantiasis. Over 120 million people are currently infected, with about 40 million disfigured and incapacitated by disease (WHO, 2012). Filariasis is endemic in 17 states and six union territories, with about 553 million people at risk of infected in India (Sabesan *et al.*, 2010). Mosquito control is complex and expensive task, frequently requiring the co-operative efforts of communities, as well as groups of industry, agriculture and state and local governments. Synthetic pesticides, often adversely impact the environment and human health and pesticide resistance, has resulted in the resurgence of mosquito borne diseases (Becker *et al.*, 2003). Today, environmental safety is considered to be of paramount importance. This has restrained the need for research and development of environmentally safe and biodegradable pesticides.

Mosquito larvae control using larvicidal agents which is a major component in the control of vector borne diseases. Thus, research against plants as potential larvicides as considered viable and preferred as an alternative in the control of the mosquito species at the society level (Evans *et al.*, 1997). Synthetic insecticides are today at the forefront of mosquito controlling agent. Furthermore, controlling the mosquitoes has become complicated despite of their resistance to these chemicals, as well as the toxicity of insecticides to fish and other non-target organisms (Wattanachai and Tintanon, 1999; Rohani *et al.*, 2001) There is an urgent need to develop new materials for controlling mosquitoes in an environmentally safe way using biodegradable and target-specific insecticides against them. Plant extracts has been suggested as alternative for insect control because some are selective, biodegrade to non-toxic products have few effects on non-target organisms and the environment (Pavela, 2007).

In view of residue problems in the environment and the evolution of insect resistance to synthetic insecticides like DDT and other chlorinated hydrocarbons, the recent trend is to explore plants to obtain extracts that are safe for non-target animals and do not pose any residue problem but are still able to repress pest populations. Though several compounds of plant origin have been described as insecticides-larvicides, there is a wide scope for the discovery of more effective plant products. Further research doubtlessly will lead the improved formulations with enhanced activity, which may in time become environmentally acceptable and replace conventional insecticides for mosquito control.

Plants would have been an another source for mosquitocidal, because they create an potential source of bio-active chemicals which is generally free harmful effects. Pyto-extracts is emerging as an potential mosquito control agents, with low-cost, easy-to-administer and risk-free properties. Herbal products has been used as an natural insecticides before the invention of synthetic organic insecticides and need to be evaluated for efficacy and safety (ICMR Bulletin, 2003). In recent years, numerous studies on plant extracts

against mosquito larvae have been conducted around the world.

In the present study experimental results of ethanol solvent extract of *Cardiospermum halicacabum* was encroached and revealed to be more toxic to immature stages of *Culex quinquefasciatus.* The early in start larvae were more susceptible than the later ones and the pupae, which was not much affected by all the solvents. This may be due to the non -feeding behavior of pupae, whereas the bio-pesticide enters the insect system through oral feeding and affects the gut and further organs. The several plants engaging biological activities suggest that plants are of worth consideration with all the control strategies involved in vector control in terms of feasibility cost effectiveness and easy availability of plant resources (Kumar *et al.,* 2012a)

In the present study 24 hrs of bioassay is a major tool for calculating the toxicity of phytotoxins, and a number of researchers have been applying this method of asses to the toxic effect of different plant extracts on mosquitoes (Patil *et al.,* 2014). In the present study the acute toxicity tests against the early fourth instars of *Culex quinquefasciatus,* larval mortality increased with increased concentrations of the extracts and same trend was observed by multiple workers with botanicals (Kumar *et al.,* 2012b; Patil *et al.,* 2014).

The ethanol extracts of plant was proven to show significant larvicidal and pupicidal activity against *Culex quinquefasciatus.* The acquired results indicates that ethanolic extract was more capable than control, which was similar observation reported by (EL-Sheikh *et al.,* 2011). These results are also comparable to earlier reports that (Murugan *et al.,* 2012) who have stated that the bio-larvicidal and pupicidal activity of *Acalypha alnifolia* against the I to IV instar. Further, Prathibha *et al.* (2011) reported that the G6 larvicidal efficacy of *Euodia ridleyi* against *C. quinquefasciatus.* Rawani *et al.* (2009) established the larvicidal properties of basic extracts of *Carica papaya, Murraya paniculata* and *Cleistanthus collinus* against *C. quinquefasciatus* and suggested that the presence of many bioactive principles for their bio-control potentiality.

Shallan *et al*. (2005) revised that progress the state of knowledge on larvicidal plants species and listed the growth and reproduction inhibiting, phytochemicals, synergistic, botanical ovicides, are additive and antagonistic combined action special effects of botanical mixtures, residual capacity and effects on non-target organisms, and appearance of resistance. Gokulakishnan *et al.* (2012) tested that larvicidal and ovicidal efficacy of different solvent leaf extracts of *A. indica* against *An. Stephensi.* That hatch rates were assessed 48h after treatment 181.00ppm, respectively. Rahuman and Venkatesan (2008) has been reported that LC_{50} value of petroleum ether extracts of *Jatropha curcas, Pedilanthus tithymaloides, Phyllanthus amarus, Euphorbia hirta,* and *Eutirucalli* were 8.79, 55.26, 90.92,272.36 at 4.25 ppm, respectively against *Ae. aegypti.*

In the present study, the ethanol extract of plant shows maximum efficacy. The dose dependent response in larval mortality were noticed in the present study. Larvicidal activities of crude extract in these solvents might have some complex mixture of biocidal active compounds, including phenolic, terpenoides, flavonoids and alkaloids which may jointly or independently contribute to produce mortality of larvae. Phytochemical limonin and nomilin from some citrus plants were reported to have larvicidal activity against mosquitoes (Bilal *et al.,* 2012). Acetone extracts constains maximum quantity of phenols and flavones, terpenoids, tannins and polyphenols (Tiweri *et al.,* 2011) and these phytochemicals are repenoids, tannins and polyphenols (Tiwari *et al.,* 2011) and these phytochemicals are reported for their insecticidal activity.

In the present study, ethanol extracts was found to be effective larvicidal and pupicidal agent. Similar results were obtained in the studies on *A. squamosal* extracts against mosquito larvae (Mehra and Hiradhar, 2000; George and Vincent, 2005). The leaf extracts of *Cassia fistula* are known to have larvicidal and ovicidal activity against *Anopheles, Aedes* and *Culex* mosquitoes (Govindarajan, 2009). The results of our study indicate that plant extracts of *Cardiospermum halicacabum* had larvicidal and pupicidal activity comparable to azadirachtin which is a proven mosquito larvicide (Modue and Nisber, 2009). Further characterization from acetone extracts of Diospyrosy compounds. This becomes alternatives to the conventional insecticides used for the control of *Culex quinquefasciatus*.

In the present investigation, larval duration was increased in treated larvae and drastic percentage reduction in larvae developing into pupae. Also, slow movement and peculiar coiling in treated larvae were observed. This may be due to the toxic substances present in the plant extracts. The present finding collaborates with the earlier findings of Mwangi and Rembold (1986) observed that the presence of lethal substances which disturbs the endocrine mechanism which regulates the moulting and metamorphosis. In general the mortality increase with increasing concentration of compounds and exposure time. The abnormalities in the metamorphosis might due to imbalance in hormones (Karmegam *et al.,* 1997) prolonged larval and pupal periods while using plant extracts have been reported by Saxena and Sexena (1992) and Daniel *et al.* (1995).

Insect growth regulators (IGRs) or third-generation insecticides (Williams, 1967; Staal, 1997) exert their insecticidal effects through their influence on development, metamorphosis and reproduction of mosquitoes by disrupting the normal activity of the endocrine system. Exposure of *Anopheles stephensi* larvae to sub-lethal doses of neem leaves extracts prolonged larval development and reduced pupal weight (Murugan *et al.,* 1996; Su and Mulla, 1999a). Several morphological abnormalities, have been observed in *Anopheles stephensis* larvae when treated with ethanol extract of *Ageratum conyzoides* (Saxena and Saxena, 1992). Larval period was not affected at lower concentrations but was lengthened up to 10 days. This failure in adult emergence could be due to insufficient availability of chitin during metamorphosis resulting in death of larvae and pupae entangled in the weak integument. Similar phyto extract induced degenerative effects on the life cycle of *Anopheles stephensi* as observed in the present have also been reported by Dhar *et al*. (1996).

There was a delay in the development of larvae to the pupal stage when the first, second, third or fourth instar larvae were exposed to plant extracts. This, may be due to the presence of high juvenile hormone levels in the larvae or else due to chemical compounds in the medicinal plant, preventing normal pupation and preventing adult emergence from occurring. Mohtar *et al.* (1999) report

the effect of a methanol -aqueous extracts of *Nerium indicum* leaf at 100 mg/I on different larval instars of *Ae. aegypti* and show an elongation of the preimago period for all their larvae treated when compared to the control.

Growth of the larvae in the treated groups decreased with the increasing concentration of the extract as compared to control groups, however there was negligible effect on the average development period of larvae in the treated groups compared to the larvae in the control groups. Visual observations suggest that the extract interfere in the developmental process inhibiting the moulting process causing "ecdysial failure". Thus moulting individuals failed to extricate themselves from the old cuticle during larval, pupal molt and some with part of the old head capsule remaining attached to the pupae as reported in several plant extracts against mosquito species (Schmutterer *et al.,* 1990). Mortality in the successfully molted pupae was visible during early stage of pupal development period as was evident from pupae dead without pigmentation and during late pupal stage till the imaginal skin became pigmented and eventually died. In the case of ovicidal activity, exposure to freshly laid eggs was more effective than to the older eggs. It has been shown that the age of the embryos at the time of reference to the effectiveness of the chitin synthesis inhibitor, dimilin to *Culex* the petroleum ether extract of *Artemisia annua* had significant influence on hatching and post-hatching development of *Anopheles stephens* (Sharma *et al.,* 2005).

The plant extracts drastically reduced the fecundity of the females only few adult survived. The highest concentration tested significantly reduced larvicides and adulticides. The neem limnoids would act as an oviposition repllent and or detterrent to *A. Stephensi* (Su and Mulla, 1999b). The larvicidal activity of crude acetone, ethyl acetate, hexane, ethonal and petroleum ether extracts of the leaf of *C. asiatica* and *Mukia scabrella* against the early fourth instar larvae of C. quinquefasciatus (Rahuman *et al*. 2009). LC_{50} of petroleum ether extracts of *Jatropha curcas, pedilanthus tithymaloides, phyllanthus amarus*. Kamaraj *et al*. (2008) have reported that the peel ethonal extracts of *C. sinensis,* leaf and flower ethyl acetale extract of *O. canum* against the larvae of *A. stephensi.* It was found that the average number of eggs laid by females that emerged from medicinal plant treatment was lower than the number of eggs laid by the females of the control group. Hatchability of these eggs was also low and the size of the first generation was small when mosquitoes were treated at the third and fourth instar larval stage, including mosquitoes treated with extracts. The present study focused on the larvicidal as well as the oviposition altering activity of crude plant extracts. The phytochemicals interfered with proper function of mitochondria more specifically at the portion transforming sites (Usta, 2002) and phytochemicals primarily effect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae (David *et al.,* 2000). The death of treated larvae may be due to the ability of the moulting bodies to swallow sufficient volume of air to split the old cuticle and expand the new one during ecdysis or to a metamorphosis inhibiting effect of plant extract which is possibly based on the disturbance of the hormonal regulation (Saxena *et al.,* 1992).

In the present study, all concentration of plant extracts used exhibited repellency activity against *Culex quinquefasciatus* females. The present study indicates that the ethanol extraction of plants was more effective in exhibiting a repellency action against the mosquito tested compared to the control. Many plant extracts and essential oils manifest repellency activity against different mosquito species. Prajapati *et al*. (2005) using essential oils extracted from 10 medicinal plants against *Anopheles stephensi* and *Culex quinquefasciatus* and Jaenson *et al*. (2006) using ethyl acetate extracts *Hyptis suaveolens* and *Rhododendon tomentosum.*

Repellents provide a mechanism for protecting human from the bites of arthropods. The desired quality in the design of an arthropod repellent includes a long-lasting repellent activity, being of low toxicity to humans and being non-irritating. Extracts (ethanol) of the indigenous plant and their different parts against the larval and adult stages of *Culex quinquefasciatus* clearly affected the various toxicology and repellent aspects used, solvent in extraction and concentration of the extract. The percentage of larval mortality increased with the extract concentration used.

On the other hand, the relent assay shows promising results, as the ethanolic extracts of *Cardiospermum halicacabum* have strong repellency effects against *Culex quinquefasciatus* and have potential as products for personal protection against the mosquitoes. The results agree with the finding of Govindarajan (2011) who have studied the larvicidal and repellent properties of essential oils from various parts of four plant species *Cymbopogan citrates, Cinnamomm zeylanicum, Rosmarinus officinalis* and *Zingiber officinale* against *Culex tritaeniorhynchus* and *A. subpictus*. Smoke emerged from *Albizzia amar* and *Ocimum basilicum* considerably affect the mosquito survival and pronounced high repellent potential (Murugan *et al.,* 2007).

References

Abbott, W.S. (1925) A method of computing the effectiveness of insecticides. *J. Eco. Ento*.,**18**: 265 – 267.

Alagarmalai Jeyasankar, Selvaraj Premalatha and Kuppusamy Elumalai (2012) Biological activities of *Solanum pseudocapsicum* (Solanaceae) against, cotton bollworm, *Helicoverpa armigera* Hübner and armyworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). *Asian Pac. J. Trop. Biomed*., **5**: 1–5.

Arivoli, S., John Ravindran, K. and Samuel Tennyson (2012) Larvicidal Efficacy of plant extracts against the malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *World J. Med. Sci*., **7(2)**: 77–80.

Arunachalam, K., Murugan, R. and Parimelazhagan, T. (2014) Evaluation of Antioxidant Activity and Nutritional and Chemical Composition of *Ficus amplissima* (Smith Fruit). *Int. J. Food Prop.*, **17**: 454 – 468.

Babu, R. and Murugan, K. (1998) Interactive effect of neem seed kerna and neem gum extract on the control of *Culex quinquefasciatus* Say. *Neem Newsletter*, **15(2)**:9-11.

Balu Selvakumar, Gokulakrishnan, J., Elanchezhiyan, K. and Deepa, J. (2015) Mosquito larvicidal, ovicidal and pupicidal activities of *Annona reticulata* linn (Annonaceae) against *Aedes aegypti* (linn.), *Anopheles stephensi* liston and *Culex quinquefasciatus* (say) (Diptera : Culicidae). *Int. J. Rec. Sci. Res*., **6(2)**: 2690 – 2696.

Becker, N., Petric, D., Zgomba, M., Boase, C., Dahl, C., Lane, J. and Kaiser, A. (2003) Mosquitoes and their control. Kluwer Academic Plenum, New York, pp. 46 – 48.

Bhattacharya, S. and Basu, P. (2016) The Southern House Mosquito, *Culex quinquefasciatus*: profile of a smart vector, *Journal of Entomology and Zoology Studies*, **4(2)**: 73-81

Bilal, A., Jahan, N., Ahmed, A., Bilal, S.N., Habib, S. and Hajra, S. (2012) Phytochemical and Pharmacological studies on *Ocimum basilicum* Linn - A Review. *I.J.C.R.R.*, **4 (23)**: 116 – 121.

Brown, A.W.A. (1986) Insecticide resistance in mosquitoes: a pragmatic review. *J. Amer. Mosq. Cont. Assoc*., **2**: 123 – 140.

Caraballo, A.J. (2000) Mosquito repellent action of Neem. J. *Am. Mosq. Control. Assoc*. **16 (1)**: 45 – 46.

Daniel, T., Umarani, S. and Sakthivadivel, M. (1995) Insecticidal action of *Ervatamia divaricata* L. and *Acalypha indica* L. against *Culex quinquefasciatus* Say. *Geobios News Report*, **14 (2)**: 95 – 98.

Das, N.G., Goswami, D. and Rabha, B. (2007) Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *J. Vect. Borne. Dis*., **44**: 145 – 148.

David, J.P., Rey, D., Pautou, M.P. and Meyran, J.C. (2000) Insecticidal, antifeedant and oviposition deterrent effects of the essential oil and individual compounds from leaves of *Chloroxylon swietenia* DC. *J. Invertebrate Pathol*.,**75** : 9 – 18.

Deshmukh, P.B. and Renapukar, D.M. (1987) Insect growth regulatory activity of some indigenous plant extracts. *Ins. Sci. Appli*., **8(1)**: 81 – 83.

Dhar, R., Dawar, H.J., Garg, S., Basir, S.F., and Pradas, S. (1996) Effect of volatiles from neem and other natural products on gonadotropic cycle and oviposition of *Anopheles stephensi* and *Anopheles culicifacies* (Diptera: Culicidae). *J. Med. Ento*., **33(2)**: 195-201.

Duncan, D.B. (1955) Multiple range and multiple 'F' tests. *Biometrics*. **11** : 1 – 42.

Elango, G., Rahuman, A.A., Bagavan, A., Kamaraj, C., Zahir, A.A. and Venkatesan, C. (2009) Laboratory study on larvicidal activity of indigenous plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. *Paras. Res*., **104(6)**: 1381-1388.

Elango, G., Rahuman, A.A., Kamaraj, C., Zahir, A.A. and Bagavan, A. (2010) Studies on effects of indigenous plant extracts on filarial vector *Culex tritaeniorhynchus* Giles. *Parasitol. Res*., **107**: 167 – 176.

El-Sheikh, T.M., Al-Fifi, Z.I. and Alabboud, M.A. (2012) Larvicidal and repellent effect of some *Tribulus terrestris* L. (Zygophyllaceae) extracts against the dengue fever mosquito *Aedes aegypti* (Diptera: Culicidae). *J. Saudi Chem. Soc*., **18**: 12 – 19.

Evans, P.H., Bowers, W.S., Sener, B., Bingol, F. and Erdogan, I. (1997) Activity of Turkish medicinal plants against mosquitoes *Aedes aegypti* and *Anopheles gambiae*. *Insect Sci. Appl*., **16**: 339 – 42.

Finney, D.J. (1971) Probit Analysis, 3rd ed. Cambridge: Cambridge university press. p. 333.

Gaston,K.J. (1994) Rarity. London: Chapman and Hall.

Ghosh, A., Chowdhury, N. and Chandra, G. (2012) Plant extracts as potential mosquito larvicides. *Indian J. Med. Res*., **135**: 581 – 598.

George, S. and Vincent, S. (2005) Comparative efficacy of *Annona squamosa* Linn. and *Pongamia glabra* Vent. to *Azadirachta indica* A. juss against mosquitoes. *J. Vector. Borne Dis*., **42(4)**:159 – 163.

Ghosh, A., Chowdhury, N. and Chandra, G. (2013) Laboratory evaluation of a phytosteroid compound of mature leaves of day jasmine (Solanaceae: Solanales) against larvae of *Culex quinquefasciatus* (Diptera: Culicidae) and non-target organisms. *Parasitol. Res*., **103**: 221 – 277.

Girdhar, G., Deval, K. and Mittal, P.K. (1988) Mosquito control by *Calotropis latex*, *J. Pesticides*, **15(2)**: 26 – 29.

Govindarajan, M. (2009) Bioefficacy of *Cassia fistula* Linn. (Leguminosae) leaf extract against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae). *Eur. Rev.Med. Pharmacol. Sci*.,**13(2)**: 99 – 103.

Govindarajan, M. (2011) Mosquito larvicidal and ovicidal activity of *Cardiospermum halicacabum* Linn. (Family: Sapindaceae) leaf extract against *Culex quinquefasciatus* (say.) and *Aedes aegypti* (Linn.) (Diptera: Culicidae). *Eur. Rev. Med. Pharmacol. Sci*., **15(7)**: 787 – 794.

Govindarajan, M., Mathivanan, T., Elumalai, K., Krishnappa, K. and Anandan, A. (2011) Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Asian. Pac. J. Trop. Biomed*.,**1(1)**: 43 – 48.

ICMR (2003) Prospect of using herbal products in the control of mosquito vector. *Indian Council of Medical Research Bulletin*, **33(1)**:10.

Jaenson, T.G., Palsson, K. and Borg Karlson, A.K. (2006) Evaluation of extracts and oils of mosquito (Diptera: Culicidae) repellent plants from Sweden and GuineaBissau. J. Med. Entomol., 43: 113 – 119.

Jeyabalan, D., Arul, N. and Thangamathi, P. (2003) Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, *A. stephensi* Liston. *Bioresour. Technol*., **89 (2)**: 185 – 189.

Kamaraj, C., Rahuman, A.A., Bhagavan, A. (2008) Screening for antifeedant and larvicidal activity of plant extracts against *Helicoverpa armigera* (Hübner), *Sylepta derogata* (F.) and *Anopheles stephensi* (Liston). *Parasitol. Res*., **103(6)**: 1361 – 1368.

Kamaraj, C., Bagavan, A., Elango, G., Zahir, A.A., Rajkumar, G. and Mariamuthu, S. (2011) Larvicidal activity of medicinal plant extracts against *Anopheles stephensi* and *Culex tritaeniorhynchus*. *Indian. J. Med. Res*., **134** : 101 – 106.

Karmegan, N., Sakthivadivel, M., Anuradha, V. and Daniel, T. (1997) Indigenous plant extracts as larvicidal agents against *Culex quinquefasciatus* Say. *Bioresource Technol*., **5**: 137–140.

Kovendan, K. and Murugan, K. (2011) Effect of medicinal plants on the mosquito vectors from the different agro-climatic regions of Tamil Nadu, India. *Adv. Environ. Biol*., **5 (2)**: 335 – 344. K

Kumar, P., Mishra, S., Malik, A. and Satya, S. (2012a) Compositional analysis and insecticidal activity of *Eucalyptus globules* (family: Myrtaceae) essential oil against housefly, *Musca domestica*. *Acta. Tropica*.,**122 (2)**: 212 – 218.

Kumar, G., Karthik, L., Rao, K.V.b., Kirthi, A.V. and, Rahuman, A.A. (2012b) Larvicidal, repellent and ovicidal activity of *Calotropis gigantea* against *Culex gelidus* and *Culex tritaeniorhynchus* (Diptera: Culicidae). *J. Agr. Tech*., **8(3)**: 869-880.

Lane, R.P. (1993) Sandflies (*Phlebotomine*). In: Lane, R.P.and Crosskey, R.W., eds. *Medical insects and arachnids*. London: Chapman and Hall, 78–119.

Lee, S.E. (2000) Mosquito larvicidal activity of pipernonaline, a piperidine alkaloid derived from long pepper, *Piper longum*. *J. Am. Mosquito Contr. Assoc*., **16** : 245 – 247.

Lotfy,W.A. (2014) Climate change and epidemiology of human parasitosis in Egypt: A review. *J.Adv. Res*., **(6)**: 607–613.

Markouk, M., Bekkouche, K., Larhsini, M., Bousaid, H., Lazrek, H.B. and Jana, M. (2001) Evaluation of some Moroccan medicinal plant extracts for larvicidal activity. *J. Ethnopharmacol*.,**73**: 293 – 297.

Mehra, B.K. and Hiradhar, P.K. (2000) Effect of crude acetone extract of seeds of *Annona squamosa* Linn. (Family: Annonaceae) on possible control potential against larvae of *Culex quinquefasciatus* Say. *J. Entom. Res*., **24** :141 – 146.

Modue and Nisber (2009) Vector competence of Australian mosquitoes (Diptera: Culicidae) for *Japanese encephalitis* virus. *J. Med. Entomol*., **40(1)** 82 – 90.

Mohtar, M., Yarmo, M.A. and Kadri, A. (1999) The effects of *Nerium inidicum* leaf extract on *Aedes aegypti* larvae. *J. Tropl. Forest Products*, **5**: 87 – 92.

Muthukrishnan, J., and Puspalatha, E. (2001) Effects of plant extracts of fecundity and fertility of mosquitoes. *J. Appl.Ento*., **125**:31-35.

Murugan, K. and Jeyabalan, D. (1999) Mosquitocidal effect of certain plant extracts on *Anopheles stephensi*. *Curr. Science*, **76(5)**: 631 – 633.

Murugan, K., Babu, T.S., Jeyabalan, D., Senthil Kumar, N. and Sivaramkrishnan, S. (1996) Antipupational effect of neem oil and neem seed kernel extract against mosquito larvae of *An. stephensi* (Liston). *J. Entomol. Res*., **20**: 137 – 139.

Murugan, K., Murugan, P. and Noortheen, A. (2007) Larvicidal and repellent potential of *Albizia amara* Boivin and *Ocimum basilicum* L. against dengue vector, *Aedes Aegypti* (Insecta: Diptera: Culicidae). *Biores. Technol*., **98**: 198 – 201.

Murugan, K., Mahesh Kumar, P., Kovendan, K., Amerasan, D., Subrmaniam, J. and Shiou H.J. (2012) Larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* orange peel extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol. Res*.,**111**: 1757 – 1769.

Mwangi, R.W and Rembold, H. (1986) Growth regulation activity of *Melia volkensil* extracts against the larvae of *Aedes aegypti*. Proc. 3rd Int. Neem Conf., Nairobi, Kenya. pp.669-681.

Naqqash, M.N., Gökçe, A., Bakhsh, A. and Salim, M. (2016) Insecticide resistance and its molecular basis in urban insect pests. *Parasitol. Res*. **115**:1363–1373.

Nisar Ahmad, Hina Fazal, Bilal Abbasi and Mazhar Iqbal (2011) In vitro larvicidal potential against *Anopheles stephensi* and antioxidative enzyme activities of *Ginkgo biloba*, *Stevia rebaudiana* and *Parthenium hysterophorous*. *Asian Pac. J. Tro. Med*., **23**: 169 – 175.

Patil, P. B., Kallapur S. V., Kallapur, V.L. and Holihosur, S. N. (2014) *Clerodendron inerme* Gaertn. Plant as an effective natural product against dengue and filarial vector mosquitoes. *Asian Pac. J. Tropical Disease*, **4(1)**: 453-462.

Pavela, R. (2007) Larvicidal effects of various Euro-Asiatic plants against *C. quinquefasciatus* say larvae (Diptera: Culicidae). *Parasitol., Res*. **36**:821–823.

Pavela R. (2015) Acute toxicity and synergistic and antagonistic effects of the aromatic compounds of some essential oils against *Culex quinquefasciatus* Say larvae. Parasitol. Res. **114**:3835–3853.

Prabhu, K., Murugan, K., Nareshkumar, A., Ramasubramanian, N. and Bragadeeswaran, S. (2011a) Larvicidal and repellent potential of *Moringa oleifera* against dengue vector, *Anopheles stephensi*, Filarial vector, *Culex quinquefasciatus* (Insecta: Diptera: Culicidae). *Asian Pac. J. Trop. Biomed.*, **1(2)**: 127 – 132.

Prajapati, V., Tripathi, A.K., Agarwal, K.K. and Khanuja, S.P. (2005) Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Bioresour. Technol*., **96(16)**: 1749 – 1757.

Prathibha, K.P., Raghavendra, B.S. and Vijayan, V.A. (2011) Evaluation of larvicidal effect of *Euodia ridleyi*, Hoch, leaf extract against three mosquito species at Mysore. *Int. J. Biol. Sci. Eng.*, **02(01)**: 106 – 10.

Rahuman, A.A. and Venkatesan, P. (2008) Larvicidal efficacy of five cucurbitaceous plant leaf extracts against mosquito species. *Parasitol. Res*., **103**: 133 – 139.

Rahuman, A., Bagavan, A., Kamaraj, C., Vadivelu, M., Zahir, A. and Elango, G. (2009) Evaluation of indigenous plant extracts against larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasit. Research*, **104**:637-643

Raju,K., Jambulingam, P., Sabesan, S. and Vanamail, P. (2010) Lymphatic filariasis in India: epidemiology and control measures. Review.*J. Postgrad. Med*. ,**56(3)**:232-8.

Raven, P.H., Evert, R.F. and Eichhorn, S.E. (1999) Families yielding important phytopharmaceuticals, 6th edition. WH Freeman, New York. Gleiser.

Rawani, A., Haldar, K.M., Ghosh, A. and Chandra, G. (2009) Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol. Res*.,**105 (5)**: 1411 – 1417.

Reegan, A. D., Munusamy,R.G., Micheal G.P. and Savarimuthu, I. (2015) Ovicidal and Oviposition Deterrent Activities of Medicinal Plant Extracts Against *Aedes aegypti* L. and *Culex quinquefasciatus* Say Mosquitoes (Diptera: Culicidae), *Osong Public Health Res Perspect*. **6(1)**: 64–69.

Reiter, P. (2001) Climate change and mosquito-borne disease. **109 (1)**:141-61.

Regnault-Roger, C., Charles Vincent and John T. Arnason (2012) Essential Oils in Insect Control: Low-Risk Products in a High-Stakes World. *Annual Review of Entomology*, **57(1)**:405-24.

Rohani, A., Chu,W.L., Saadiyah, I., Lee, H.L. and Phang, S.M. (2001) Insecticide resistance status of *Ae. albopictus* and *Ae. aegypti* collected from urban and rural in major towns of Malaysia. *Trop. Biomed*., **18(1)**: 29 – 39.

Sabesan, S., Vanamail, P., Raju, K. and Jambulingam, P. (2010) Lymphatic filariasis in India: epidemiology and control measures. *J. Postgrad. Medic*., **56(3)**: 232.

Saxena, A. and Saxena, R.C. (1992) Effect of *Agertum conyzoides* extract on the developmental stages of malaria vector, *Anopheles stephensi* (Diptera: Culicidae). *Journal of Environmental Biology*, **13(3)**: 207-209.

Saxena, R.C., Dixit, O.P. and Sukumaran, P. (1992) Laboratory assessment of indigenous plant extracts for anti-juvenile hormone activity in *Culex quinquefasciatus*. *Indian J. Med. Re*., **95**: 199-204.

Schmutterer, H. (1990) Properties and potential of natural pesticides from the neem tree, *Ann. Review Entomol.*, **35**: 165 – 271.

Seyoum, E., Bateman, R. P. and Charnley, A. K. (2002) The effect of *Metarhizium anisopliae* var *acridum* on haemolymph energy reserves and flight capability in the desert locust, *Schistocerca gregaria*. *J. App. Ento*., **126**: 119-124.

Shallan, E.A.S., Canyonb, D., Younesc, M.W.F., Abdel Wahaba, H., and Mansoura, A.H. (2005) A review of botanical phytochemicals with mosquitocidal potential. *Environ. Int*., **3**: 1149 – 1166.

Sharma, P., Mohan, L. and Srivastava, C.N. (2005) Larvicidal potential of *Nerium indicum* and *Thuja orientalis* extracts against malaria and Japanese encephalitis vector. *J. Environ. Biol*., **26**: 657 – 660.

Sharook, A.Z.M., Balan, Y.J. and Rembold, H.J. (1991) Insect growth inhibitor from two tropical Meliaceae effect of crude extracts on mosquito larvae. *J. Appl.d Entomology*, **111**: 425 – 430.

Sharma, R.N., Gupta, A.S., Patwardhan, S.A., Hebbalker, D.S., Tare, V. and Bhonde, S.B. (1992) Bioactivity of lamiaceae plants against insects. *Indian J. Exp. Biol*., **30**: 244 – 246.

Shyampada Mandal, S. (2011) Repellent activity of Eucalyptus and *Azadirachta indica* seed oil against the filarial mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) in India. *Asian Pac. J. Trop. Biomed*., **1**: 181 – 184.

Silva, A.G., Almeida, D.L., Ronchi, S.N., Bento, A.C., Scherer, R., Ramos, A.C. and Cruz, Z.M. (2010) The essential oil of Brazilian pepper, *Schinus terebinthifolia* Raddi in larval control of *Stegomyia aegypti* (Linnaeus, 1762). *Parasit. Vectors*, **3**: 79 – 73.

Staal, G.B. (1977) Insect control with insect growth regulators based on insect hormones, pp. 253-283.

Su, T., and Mulla, M.S. (1999a) Oviposition bioassay responses of *Culex tarsalis* and *Culex quinquefasciatus* to neem products containing azadirachtin. *Entomologia Experimentalis et Applicata*, **91**:337-345.

Sukumar, K., Perich, M.J. and Boobar, L.R. (1991) Botanical derivatives in mosquito control: a review. *J. Am. Mosq. Control. Assoc*., **7(2)**: 210 – 237.

Supavarn, P., Fred, W.K.I. and Roy, S. (1974) Phytochemical extracts for control of mosquito larvae. *Mosque. News*, **34**: 404 – 408.

Sweta, B., Lalit Mohan and Chand Narayan Srivastava (2015) Efficacy of *Cuscuta reflexa* extract and its synergistic activity with Temephos against mosquito larvae. *I.J.M.E.R*., **2(1)**: 34 – 41.

Tiwari, V.K., Kishore, N, Mishra, B.B. and Tripathi, V. (2011) A review on natural products with mosquitosidal potentials. In: Tiwari, V.K. editor. Opportunity, challenge and scope of natural products in medicinal chemistry. *Research Signpost*, p. 335 – 65.

Thangam, T.S. and Kathiresan, K. (2011) Synergistic effects of insecticides with plant extracts on mosquito larvae. *Trop. Biomed*., **7**:135–7.

Usta, J., Kreydiyyeh, S., Bakajian, K. and Nakkash-chmaisse, H. (2002) In vitro effect of eugenol and cinnamaldehyde on membrane potential and respiratory complexes in isolated rat liver mitochondria. *Food and Chemical Toxicol*., **40**: 935 – 940.

Vincent, O., Nyasembe, Xavier Cheseto, Fatma Kaplan, Woodbridge, A.. Foster, Peter, E. and Teal. A. (2015) The invasive American weed *Parthenium hysterophorus* can negatively impact malaria control in Africa. *Plos one*, **10(9)**: 153 – 157.

Venkatachalam, M.R. and Jebanesan, A. (2001) Repellent activity of *Ferronia elephantum* Corr. (Rutaceae) leaf extract against *Aedes aegypti*. *Bioresour. Technol*., **76 (3)**: 287 – 288.

Wattanachai, P. and Tintanon, B. (1999) Resistance of *Ae. aegypti* to chemical compounds in aerosol insecticide products in different areas of Bangkok, Thailand. *Commun. Dis. J*., **25**: 188 – 191.

Williams, C.M. (1967) Third-generation pesticides. *Scientific American*, **217**:13-17.

WHO (1996) Report of the WHO informal consultation on the evaluation and testing of insecticides. WHO, Geneva, pp 9, 32-36, 50-52.

World Health Organization, (2012) Dengue and severe dengue. http://www.who.int/mediacentre/factsheets/fs117/en/

Yakob, L. Rebecca Dunning and Guiyun Yan (2011) Indoor residual spray and insecticide-treated bednets for malaria control: theoretical synergisms and antagonisms, *J.R.Socity Interface*, **8(59)**: 799–806.