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Studies on Mosquitocidal and biological activity of endemic plants of Nilgiris Hills against filarial vector, *Culex quinquefasciatus* (Say) (Insecta: Diptera: Culicidae)

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Abstract

The methanol leaf extracts of *Glochidion neilgherrense*, *Cinnamomum wightii* and *Leucas linifolia* are highly toxic even at low doses proven to be useful for larvicidal, pupicidal and adulticidal activity against *Culex quinquefasciatus*. The extraction of *Glochidion neilgherrense*, *Cinnamomum wightii* and *Leucas linifolia* are dose dependent and the mortality of the larvae increases as the doses of the sample were increased. Among all the instars, the fourth instar larvae were less susceptible to the plant extracts than first instar larvae. The mortality caused by some neural and muscular disturbance by the presence of variety of active compounds such as cytotoxic diterperiods, lactones and flavonoids in the plants. It was observed that the increase in concentration of extracts increased the potential activity of biocides against *Culex quinquefasciatus*. The decrease in egg hatchability was found to be dose dependent. There was increase in the larval-pupal duration, developmental duration, decrease in fecundity and egg hatchability, biting deterrency, in our study. The extract showed ovipositional deterrence and effective repellence against *Culex quinquefasciatus* at different concentration, with the observation on that maximal egg were laid in lower concentration of the extract and control. In oviposition deterrent and gravid mortality assay, the OAI values also indicated that the gravid and oviposited females were repelled by extracts and the reduced oviposition was due to the greater mortality of adults before they oviposited, caused by treatment of plant extract which contained of variety of active compounds. All the concentration of plant extracts hared promising mosquito repellency properties when tested against the adult mosquitoes of *Culex quinquefasciatus*. In the biting deterrency results, increasing in the concentration of plant extracts from 1% to 4% was found to increase the biting deterrency percentage.

Keywords: *Culex quinquefasciatus, Glochidion neilgherrense, Cinnamomum wightii, Leucas linifolia,* Mortality, Biology, egg hatchability, repellency, bitting detterency

Introduction

Mosquito transmit diseases like malaria, dengue, filariasis accounted for global mortality and morbidity with increased resistance to common insecticides. Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. WHO has declared mosquitoes as “Public enemy number one”. Mosquitoes borne disease are prevalent in more than 100 countries across the world, infection over 700,000,000 people every year globally and 40, 000, 0000 of Indian population. Mosquitoes are the major vector for transmission of life threatening disease like malaria, Yellow fever, dengue fever, chikungunya fever, filiariasis, encephalitis, West Nile virus infection, etc., in almost all tropical and subtropical countries and many other parts of the world (Govindrajan *et al.,* 2011; Ramar *et al.,* 2013).

Culex quinquefasciatus is a medium, light brown mosquito, the abdominal sternites of females are pale scaled with a few dark scaled patches medially. *Culex quinquefasciatus,* is a vector of lymphatic filiariasis which is widely distributed tropical disease and there are nearly 1,100 million people living in areas endemic for lymphatic filiariasis and exposed to their risk of infection; there are 102 million cases of filiariasis, either having patent microfilareaimeia or chronic filarial disease (Michael *et al.,* 1996),*Wuchereria bancrofti* accounts for approximately 90% of all filiariasis cases in the world, followed by *Brugia malayi* and *Brugia timori*. India contributes about 40% of the total global burden of filiariasis and counts for about 50% of the people at risk of infection. Recent estimates have shown that in India, 22 states were found to be endemic for filariasis and nine states (Andhra Pradesh, Bihar, Gujarat, Kerala, Maharashtra, Orissa, Tamil Nadu, Uttar Pradesh and West Bengal) contributed to about 95% of the total burden of filiariasis.

One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Further, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which act concerntly on both behavioral and physiological processes (Rawani *et al.,* 2014). Thus there is very little chance of pests developing resistance to such substances. Identifying bio-insecticides are efficient, as a well as being suitable and adaptive to ecological condition, is imperative for continued effective vector control management. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future many acts as suitable alternative product to fight against mosquito borne diseases.

The extensive use of synthetic organic insecticides during the last few decades has resulted in environmental hazards and also in the developmental of physiological resistance in most vector species. This has necessitated the need for research and development of environmental safe, biodegradable, low cost indigenous methods for vector control which can be used with minimum care by individual and communities in specific situations (Singh *et al.,* 2006). Due to the problem of pollution and vector resistance, safe plant products are being tested around the world as pest control agents (Ramaswamy and Mohan, 2014; Bagavan *et al.,* 2010).

Plant based products has been revived because of the development of resistance, cross-resistance and possible toxicity hazards associated with synthetic insecticides, bioaccumulation and pollution. Phytochemicals obtained from huge diversity of plant species are the major sources for safe and biodegradable chemicals, which can be screened for mosquito repellent and insecticidal activities (ICMR, 2003).

Botanicals are basically secondary metabolites that serve as a means of defense mechanism of the plants to with stand the continuous selection pressure form herbivore predator and other environmental factors. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities (Shaalam *et al.,* 2005). Insecticidal effects of plant extraction vary not only according to plant species, mosquito species, geographical varieties and parts used, but also due to extraction methodology adapted and the polarity of the solvents used during extraction. Phytochemicals have a major role in mosquito control programs. The bioactive plant ingredients can be obtained from the whole plant or from a specific part by extraction with different types of polar and non-polar solvents, such as petroleum ether, benzene, chloroform, methanol, absolute alcohol and acetone etc. A wide selection of plant from herbs, shrubs and large trees was used for extraction of mosquito toxins. Phytochemicals were extracted either from the whole body of little herbs of from various parts like fruits, leaves, stems, barks and roots etc., of large plants or trees. In all cases where the most toxic substances were concentrated upon, found and extracted for mosquito control.

Phytochemicals derived from plant sources can act as a means to act as larvicides, insect growth regulators, repellents and oviposition attractants and can play an important role in the interruption of the transmission of mosquito- borne disease at the individual as well as at the community level (Govindarajan *et al.,* 2008a; Nathan *et al.,* 2006).The active principles in medicinal plants are chemical compounds known as secondary plant products. Some secondary products discourage herbivores, other inhibit bacterial or fungal pathogens.

Two major categories of these compounds are alkaloids and glycosides.

More than 3,000 alkaloids have been identified in 4000 plant species' most occur in herbaceous dicots and also in fungi. Alkaloids contain nitrogen, they are usually alkaline (basic) and they have a bitter taste. Their most pronounced actions are on the nervous system, where they can produce physiological effects. The difference between a medicinal and a toxic effect of many alkaloids is often a matter of dosage (Levetin and McMahon, 2003; Guerro *et al.,* 2014). Mosquitocidal activities of various herbal products from edible crops, ornamental plants, trees, shrubs, herbs, grasses and marine plants according to the extraction procedure developed in different solvent systems and the nature of mosquitocidal activities against different life stages of different vector species act as a ready reference for further studies.

In nature, essential oils play an important role in the protection of the plants as anti-bacterials, antivirals, antifungals, insecticides and also against herbivores by reducing their appetite for such plants. Essential oils being complex mixtures of volatile organic compounds are generally produced as secondary metabolites in plants. They are constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones and phenols). Besides toxic and repellent properties, essential oils have been shown to have a pronounced effect on the developmental period, growth, adult emergence, fecundity, fertility and egg hatching of insects (Shallam *et al.*, 2005; Elango *et al.*, 2010a). Current control is based on removing the standing water in which the larvae have hatched. If removing the stagnant water is not possible, people may be able to treat it with an insecticide or with oil that covers the surface (Caron, 1996).

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in mosquito control were in use since 1920s (Shahi *et al.*, 2010), but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme. After facing several problems due of injudicious and over application of synthetic insecticides in nature, re-focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Since, the search for new bioactive compounds from the plant kingdom and an effort to determine its structure and commercial production has been initiated. At present phytochemicals make upto 1 percent of world's pesticide market (Isman, 1997).

Therefore in the present study I have screened three endemic plants *Glochidion neilgherrense*, *Cinnamomum wightii* and *Leucas linifolia* leaves extract on the larvicidal, pupicidal, adulticidal, larval, pupal and adult duration, reproductive activity, repellency and biting deterrency of *Culex quinquefasciatus.* The possible result of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive compounds from indigenous endemic medicinal plant source.

Materials and Methods

Collection and preparation of plant extracts

Healthy leaves of *Glochidion neilgherrense*, *Cinnamomum wightii* and *Leucas linifolia* were collected from Nilgiri hills of Tamilnadu, India. The plants were identified with the help of experts in the Department of Botany, Govt. 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 methanol solvents at room temperature for 3 days and filtered. The combined filtrate were concentrated to dryness by rotary evaporation at 50^{o}c and kept in a freezer. In preparing test concentrations, each plant extract were volumetrically diluted in methanol.

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/moulted larvae were used for the bioassay tests. The required quantity of different 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 methanol 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 (Finnely, 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 extracts. The paper was keep inside the beaker. Muslin cloth covering the beaker was also treated. Control insects were exposed only to distilled water with methanol solvent treated paper and muslin cloth. Mortality count was taken after 24h (Sharma *et al*., 1992).

Ovipositional assay

Different quantities of plant 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 *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:

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

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 methanol solvent were used (Sharma *et al*., 1992).

Repellency activity

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

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 methanol, distilled water with methanol solvent served as control. Different concentration of the plant extracts was applied. The control and treated arms was introduced simultaneously into the cage. The numbers of bites was count over 5 minute from 6 pm to 6 am. The experiment was conducted five times. The percentage protection was calculated by using formula:

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

[illegible]

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.

Results

Totally three locally grown different endemic medicinal plants were collected and the solvents extracts of their leaves were tested for larvicidal, pupicidal, adulticidal, larval duration, pupal duration, adult duration, reproductive activity, repellency and biting deterrency of *Culex quinquefasciatus*. The assay of the investigated plant species were carried out using different concentration with methanol on *Culex quinquefasciatus.* The plants were 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.

The LC_{50} and LC_{90} concentration of *Glochidion neilgherrense*, *Cinnamomum wightii* and *Leucas linifolia* with methanolic leaf extracts (Table 1 to 3). The result reveals that, *Leucas linifolia* requires less concentration to bring out 50% and 90% mortality of *Culex quinquefasciatus* larval forms and the LC_{50} and LC_{90} values of the experimental plant for 1st instar larval forms follows this order *Lavendula angustifolia* (0.72%; 2.07%), *Cinnamomum wightii* (1.17%; 4.56%), and *Glochidion neilgherrense* (1.64%; 5.64%). It can be clearly understood by this observation that *Lavendula angustifolia* exhibits higher mortality at lower concentration, than other experimental plants. Similar observation is registered for 2nd, 3rd and 4th instar larval forms. When comparing between the solvents, it can be clearly seen that, methanol leaf extract exhibits higher mortality at low concentration than control.

Table 1. LC_{50} and LC_{90}values of methanol leaf extracts of *Glochidion neilgherrense* against larvae of *Culex quinquefasciatus*

Mosquito Instar stages	LC_{50} (%)	LC_{90} (%)	95% confidence limit				$\chi^2(df)$	Regression equation
			LC_{50}		LC_{90}			
			LCL	UCL	LCL	UCL		
1st Instar	1.64	5.64	0.90	2.91	3.12	33.07	0.25(3)	Y=-.520+2.399
2nd Instar	1.87	6.22	1.06	3.35	3.44	35.73	2.04(3)	Y=-.667+2.454
3rd Instar	2.21	7.31	1.42	3.59	4.27	37.11	7.73(3)	Y=-.847+2.464
4th Instar	2.66	8.72	1.78	4.28	5.14	39.96	11.07(3)	Y=-1.058+2.488X

LC_{50}, LC_{90} = Lethal Concentration, LCL = Lower Confidence Limit, UCL = Upper confidence Limit, χ^2 = *Chi*-square value,*df*= *degree of freedom,* Significant at P≤0.05, PROBIT = Intercept + BX (Covariates X are transformed using the base 10.00 logarithm.

Table 2. LC_{50} and LC_{90}values of methanol leaf extracts of *Cinnamomum wightii* against larvae of *Culex quinquefasciatus*

Mosquito Instar stages	LC_{50} (%)	LC_{90} (%)	95% confidence limit				$\chi^2(df)$	Regression equation
			LC_{50}		LC_{90}			
			LCL	UCL	LCL	UCL		
1st Instar	1.17	4.56	0.64	1.81	2.71	16.80	10.70(3)	Y=-.150+2.171X
2nd Instar	1.35	5.17	0.76	2.15	2.99	21.51	11.94(3)	Y=-.289+2.201X
3rd Instar	1.58	6.15	0.93	2.53	3.51	25.76	11.55(3)	Y=-.429+2.168X
4th Instar	1.84	7.21	1.17	2.89	4.17	26.47	10.09(3)	Y=-.574+2.162X

LC_{50}, LC_{90} = Lethal Concentration, LCL = Lower Confidence Limit, UCL = Upper confidence Limit, χ^2 = *Chi*-square value,*df*= *degree of freedom,* Significant at P≤0.05, PROBIT = Intercept + BX (Covariates X are transformed using the base 10.00 logarithm

Table 3. LC_{50} and LC_{90} values of methanol leaf extracts of *Leucas linifolia* against larvae of *Culex quinquefasciatus*

Mosquito Instar stages	LC_{50} (%)	LC_{90} (%)	95% confidence limit				$\chi^2(df)$	Regression equation
			LC_{50}		LC_{90}			
			LCL	UCL	LCL	UCL		
1st Instar	0.72	2.07	0.61	0.82	1.76	2.56	1.96(3)	Y=.403+.267X
2nd Instar	0.86	2.95	0.73	0.99	2.47	3.73	2.71(3)	Y=0.155+2.397
3rd Instar	1.05	4.01	0.90	1.21	3.29	5.19	5.10(3)	Y=-0.47+2.203X
4th Instar	1.27	5.08	0.87	1.74	3.36	11.02	5.66(3)	Y=-.224+2.134

LC_{50}, LC_{90} = Lethal Concentration, LCL = Lower Confidence Limit, UCL = Upper confidence Limit, χ^2 = *Chi*-square value,*df= degree of freedom,* Significant at P≤0.05, PROBIT = Intercept + BX (Covariates X are transformed using the base 10.00 logarithm.

Totally three plant with methanolic extract of *Glochidion neilgherrense*, *Cinnamomum wightii* and *Leucas linifolia* were screened for their pupal mortality (%), adult mortality (%) and adult emergence (%), shown in Table 4. It was profound to note that, all the plant extract showed promising effect on pupae and adult. It is interesting to note that, even the methanolic extracts showed dose dependent relationship *ie.*, direct relation to pupal mortality (%) and adult mortality (%) and indirect relationship with respect to adult emergence (%). Thus the following trend of mortality percentage was observed in pupae and adult at all stages among the selected plants *Leucas linifolia* > *Cinnamomum wightii* > *Glochidion neilgherrense* and within the experimental conditions showed remarkable result of dose response of increased mortality with increase in concentration of the extracts in the solvent.

Table 4. Effect of methanol extracts of plants on the pupa and adult of *C. quinquefasciatus*

S. No	Treatment	Concentration (%)	Pupal mortality (%)	Adult mortality (%)	Adult emergency (%)
1	**Control**		00 [j]	00 [h]	100[a]
2	***Glochidion neilgherrense***	1	26[i]	24[g]	80[b]
		2	38[h]	36[f]	58[d]
		4	55[f]	52[e]	36[f]
3	***Cinnamomum wightii***	1	44[g]	40[f]	65[c]
		2	61[e]	58[d]	45[e]
		4	85[c]	83[b]	24[g]
4	***Leucas linifolia***	1	66[d]	71[c]	49[e]
		2	90[b]	86[b]	27[g]
		4	100[a]	98[a]	10[h]

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

Results of laboratory testing for three plant extract of *Glochidion neilgherrense*, *Cinnamomum wightii* and *Leucas linifolia* were screened for adult repellency (%) and ovipositional deterrency (%). It was very interesting to note that in comparision with all our treated groups, methanolic extract showed the highest adult repellency (%) and ovipositional deterrency (%). In respect to adult repellency (%), *Glochidion neilgherrense* recorded the least activity among all the concentration (14%, 24% and 34%) followed by this *Cinnamomum wightii* of 27%, 40% and 62%, and *Leucas linifolia* of 48%, 61% and 88% respectively with increasing concentration of 1%, 2% and 4% respectively (Table 5). Similar observation in respect to concentration gradient was observed for ovipositional deterrency (%) for methanolic extract (Table 5).

Table 5. Effect of methanol extracts on adult repellency and oviposition deterrency of *C. quinquefasciatus*

S. No	Treatment	Concentration (%)	Adult repellency (%)	Ovipositionl deterrency (%)
1	**Control**		00[h]	00[g]
2	***Glochidion neilgherrense***	1	14[g]	25[f]
		2	24[f]	37[e]
		4	34[e]	54[d]
3	***Cinnamomum wightii***	1	27[f]	37[e]
		2	40[d]	51[d]
		4	62[b]	74[b]
4	***Leucas linifolia***	1	48[c]	55[d]
		2	61[b]	69[c]
		4	88[a]	96[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. All the three plants acetone extracts of *Glochidion neilgherrense*, *Cinnamomum wightii* and *Leucas linifolia* screened (Table 6). Summatively it was observed that the larvae took more time to develop into pupae in all the treatments when compared to the untreated groups - control. *Glochidion neilgherrense* and *Cinnamomum wightii* exhibited comparatively reduced activity than *Leucas linifolia*. At maximum concentration of 4% with all 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 6. Larval duration of *Culex quinquefasciatus* after the treatment of methanol extracts of plants

S. No	Treatment	Concentrations (%)	Total larval duration (days)			
			1st Instar	2nd Instar	3rd Instar	4th Instar
1	**Control**		1.6[h]	2.9[g]	3.1[f]	3.6[e]
2	***Glochidion neilgherrense***	1	3.2[g]	3.9[f]	4.3[e]	5.6[d]
		2	3.5[f]	5.0[e]	5.4[d]	6.0[d]
		4	4.0[e]	6.4[d]	6.9[c]	7.3[c]
3	***Cinnamomum wightii***	1	6.0[d]	6.3[d]	6.8[c]	7.2[c]
		2	6.6[c]	6.9[c]	7.3[b]	7.6[bc]
		4	7.2[b]	7.7[b]	8.4[a]	8.9[a]
4	***Leucas linifolia***	1	6.6[c]	6.9[c]	7.2[b]	7.5[bc]
		2	7.0[b]	7.3[b]	7.6[b]	7.9[b]
		4	7.7[a]	8.0[a]	8.7[a]	9.3[a]

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

The developmental metamorphosis for pupae and adult developmental duration (days) was recorded for the plants with methanol extracts of *Glochidion neilgherrense*, *Cinnamomum wightii* and *Leucas linifolia* (Table 7). Anaysis of methanolic extract with total pupal duration (days) revealed the fact that *Glochidion neilgherrense* had lower total pupal duration of 6.7 days, whereas *Cinnamomum wightii* and *Leucas linifolia* recorded 9.0 and 9.5 days at 4% concentration. 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 quinquefasciatusie.*, exposure to plant extracts to treated groups exposed the gradual increase in pupal duration and decrease in adult longevity.

Table 7. Pupal and adult duration of *Culex quinquefasciatus* after the treatment of methanol extracts of plants

S. No	Treatment	Concentrations (%)	Total Pupal duration (days)	Total Adult duration (days)
1	**Control**		3.1[h]	71[a]
2	***Glochidion neilgherrense***	1	4.0[g]	56[b]
		2	5.2[f]	47[b]
		4	6.7[d]	28[e]
3	***Cinnamomum wightii***	1	5.7[e]	37[d]
		2	7.0[d]	24[ef]
		4	9.0[b]	09[g]
4	***Leucas linifolia***	1	6.0[e]	33[d]
		2	7.5[c]	20[g]
		4	9.5[a]	05[g]

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

Action of methanol extracts of *Glochidion neilgherrense*, *Cinnamomum wightii* and *Leucas linifolia* 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 methanol extract of plants (Table 8). Percent hatch of eggs placed in control was 100 %, whereas in 1 %, 2% and 4% concentrations of methanol extracts of *Glochidion neilgherrense* was 78 %,60 % and 48% respectively, in *Cinnamomum wightii* it was observed to be 63%, 43% and 31% respectively and least activity was recorded in *Leucas linifolia* as 47%, 25% and 12% respectively, which is a satisfying result to reveal that *Lavendula angustifolia* at 1%, 2% and 4% concentration shows promising decreased level of egg hatchability. It is also observed that as the concentration increased the egg hatchability decreased in extracts of solvents within the experimental condition in agreement with methanol extract the level of egg hatchability was highly decreased significantly with increase in concentration (Table 8).

Table 8. Effect of methanol extract on fecundity and egg hatchability of *C. quinquefasciatus*

S. No	Treatment	Concentration (%)	Fecundity (No. of eggs)	Eggs hatchability (%)
1	**Control**		204 [a]	100 [a]
2	***Glochidion neilgherrense***	1	200[a]	78 [b]
		2	189[b]	60[c]
		4	170[d]	48[d]
3	***Cinnamomum wightii***	1	182[c]	63[c]
		2	172[d]	43[d]
		4	151[e]	31[e]
4	***Leucas linifolia***	1	165[d]	47[d]
		2	153 [e]	25[f]
		4	131[g]	12[g]

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

The total number of eggs laid in ovitraps containing the given concentration of methanol extracts of plants was always less than the control. Adult fecundity also was marked decreased by the plant extracts treatment. Among the total number of eggs laid, in control 204 eggs were laid but in the treatments when placed with ovitraps with 1%, 2% and 4% acetone extracts of *Leucas linifolia* collected were 165, 153 and 131, *Cinnamomum wightii* were 182, 172, 151, *Glochidion neilgherrense* with 200, 189, 170 respectively. The plant extracts drastically reduced the fecundity of females and only adults survived, whereas the adult emergence was also significantly low in our treatment groups when compared to the control subjected to experimental conditions.

In our present study, we enumerated the larval-pupal intermediate (%) of *Culex quinquefasciatus* with various increasing concentration (1%, 2% and 4%) of methanolic extracts of three plants *Glochidion neilgherrense*, *Cinnamomum wightii* and *Leucas linifolia* and represented data in table form (Table 9).The methanol extracts of *Glochidion neilgherrense* showed that the least larval-pupal intermediate, followed by *Cinnamomum wightii* and highest was recorded in *Leucas linifolia* at experimental concentration 1%, 2% and 4% concentration with values of 47, 53 and 67 respectively.

Table 9. Effect of methanol extract of plants on larval-pupal intermediate of *C. quinquefasciatus*

S. No	Treatment	Concentration (%)	Larval- Pupal intermediate (%)
1	Control		00^{h}
2	*Glochidion neilgherrense*	1	10^{g}
		2	20^{f}
		4	37^{d}
3	*Cinnamomum wightii*	1	27^{e}
		2	35^{d}
		4	54^{b}
4	*Leucas linifolia*	1	47^{c}
		2	53^{b}
		4	67^{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 solvent against *Culex quinquefasciatus* was examined for biting deterrency (Table 10). In this observation, all the crude extracts of methanol solvent 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.

Table 10. Effect of methanol extracts of plants on biting deterrency of *C. quinquefasciatus*

S. No	Treatment	Concentration (%)	Biting detterrency (%)
1	Control		00^{h}
2	*Glochidion neilgherrense*	1	13^{g}
		2	22^{f}
		4	32^{e}
3	*Cinnamomum wightii*	1	26^{f}
		2	39^{d}
		4	60^{b}
4	*Leucas linifolia*	1	48^{c}
		2	59^{b}
		4	86^{a}

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

Discussion

Plants could be an alternative source for mosquitocidal because they constitute a potential source of bioactive chemicals and are generally free from harmful effects. Phyto-extracts are emerging as potential mosquito control agents, with low-cost, easy-to-administer and risk-free properties.Herbal products have been used as natural insecticides before the discovery of synthetic organic insecticides and need to be evaluated for efficacy and safety (ICMR, 2003). In recent years, many studies on plant extracts against mosquito larvae have been conducted around the world.

In the present study experimental results of methanol solvent extracts of *Glochidion neilgherrense*, *Cinnamomum wightii* and *Leucas linifolia* were accessed and revealed to be more toxic to immature stages of *Culex quinquefasciatus.* The early instar 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 behaviour of pupae, whereas the bio-pesticide enters the insect system through oral feeding and affects the gut and other organs. These results are also comparable to earlier reports of Murugan *et al.* (2012) who have reported that the bio-larvicidal and pupicidal activity of *Acalypha alnifolia* against the I to IV instar larvae and pupae of *C. quinquefasciatus* mosquitoes with LC_{50} value of I instar was 5.388%, II instar was 6.233%, III instar was 6.884%, IV instar was 8.594% and pupae was 10.073%. Further, Prathibha *et al.* (2011) have reported the larvicidal efficacy of *Euodia ridleyi* against *C. quinquefasciatus*. Rawani *et al.* (2009) established the larvicidal properties of crude extracts of *Carica papaya*, *Murraya paniculata* and *Cleistanthus collinus* against *C. quinquefasciatus* and suggested that the presence of many bioactive principles such as steroids, alkaloids, terpenes, saponins, etc. which may be responsible for their bio-control potentiality.

In the present study, the crude methanol extracts from plants displayed larvicidal activity at varying levels. Previous investigations *of L. stellatus* indicated that the crude extracts form the stem and root barks to have *in vitro* antimalarial activity, as well as weak toxicity against brine shrimp (BST) larvae (Nkunya *et al.*, 2000). Among the compounds isolated from the extracts was insect juvenile hormone III (JH III), which was previously isolated from this and other plant species (Toong *et al.*, 1988). The occurrence of JH III in plants has been quite intriguing since normally the compound is metabolized by insects in order to regulate their developmental process (metamorphosis). Therefore, the compound when produced by plants may have similar roles, suggesting that the plants would be producing the compound in order to deter insect accumulation, as the insects would not prefer to acquire additional JH III doses beyond what is normally required for metabolism. Accumulation of this compound beyond biochemically allowable levels would disrupt the insects' development process. Hence, the compound would act as bio-insecticide. Therefore, the presence of this compound in plant extracts would make the extracts act as readily biodegradable environmental larvicides.

Generally, the mortality percent was increased with an increase in concentration and to some extent with exposure of time. This largely attributed to increased levels of active ingredients in higher doses, as reported from other plants by several authors (EI Tayeb *et al.*, 2012; Edriss *et al.*, 2012). Regarding the effect of exposure time, the results showed that the mortality of most extracts concentrations increased slightly with the progress in time from 24 hours to 72 hours treatments. This may prove the delayed effects of botanical extracts which pertained to their mode of action with mostly through stomach route rather than contact effect (Schmuttere, 1990). Mullai *et al.* (2008) sated that the mortality effects of some cucurbitaceous extracts against *Anopheles stephensi* reached 60% and 100% mortalities after 48 hours and 72 hours treatments, respectively.

The potential of these extracts either having a larvicidal or insecticidal activity has earlier been reported by various authors. Also, many authors have widely reported the chemotherapeutic ability of some of these extract either as malaria herbs or other medicinal uses (Abdelouabeb *et al.*, 2009; Umar *et al.*, 2007). Previous study of phytochemical screening results indicated that the leaves extracts of these plants were rich in alkaloids, flavonoids and tannins, saponins which may be responsible for the insecticidal properties observed in these plants. These phytochemical have earlier been reported to have larvicidal and insecticidal abilities (Sofowora, 1993). Neem crude extract or oil has specifically been reported to inhibit metamorphosis thereby disallowing pupation or adult emergent of the mosquito (Kabaru and Gichia, 2001). The result of this study agreed with the finding of Okumu *et al.* (2007) where it was reported that neem is highly toxic to mosquito and delay pupation. Exposure of *A. gambiae* larvae to sub-lethal doses of neem and catnip leaves extract in the laboratory prolonged larvae development and pupation (Su and Mulla, 1999b).

In the present study, a significant decrease in the percentage of larval pupation was found with all plant extracts tested. Moreover, the pupation was found to depend on the plant and the solvent used for extraction. The present study showed that plant extracts had also a toxicity effect on pupae. In addition, almost all the plant extracts induced a reduction in the percentage of emerging adults from pupae produced form treated larvae. AI Dakhil and Morsy (1999) are using the neem, *Azadirachta indica* extract against *Culex pipiens* larvae and Nathan *et al.* (2005b) using methanol extracts of leaves and seeds of *Melia azadaracts* against *Anopheles stephensi* larvae.

The plant tested in the present study is known to be non-toxic to vertebrates. Moreover, it has been clearly proved that crude or partially purified plant extracts are less expensive and highly efficient for the control of mosquitoes rather than the purified compounds or extracts (Cavalcanti *et al.,* 2004; Jaenson *et al.,* 2006). Our results showed high bioactivity of the different extracts form the plant which is widely common in India. Such results may offer an opportunity for developing alternatives to rather expensive and environmentally hazardous organic insecticides. Results of the mortality, biology, repellency and biting deterrence effects of the present plant extracts on *Culex quinquefasciatus* as discussed latter confirm their potential for control of the mosquito population.

In the present study, the growth of *Culex quinquefasciatus* was remarkably affected by the plant extracts tested. It decreased as the concentration of the extract increased. Retardation in growth was induced by methanol extract tested. Such results are in agreement with earlier studies which was induced by methanol extract tested. Such results are in agreement with earlier studies using different plant extracts against different mosquito species (Jeyabalan *et al.,* 2003; Shaalan *et al.,* 2005; Nathan *et al.,* 2006).

Inhibition of growth was detected with the extracts since all larvae grew to become pupae and subsequently adults. Earlier report (Bagavan *et al*., 2008) reveals that the ethyl acetate extracts of *A. aspera* showed larvicidal activity against the early fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus*. Either difference33.s in susceptibility between mosquito species (Sukumar *et al.,* 1991) or variations in the composition of the extracts due to extraction method may explain the observed differences.

The extracts of three plants were screened for their oviposition activity against *Culex quinquefasciatus*. The plants methanol extracts showed 86% deterrency, thereby methanolic extract proven to show maximum deterrent activity than control. Among all plant with the exception of *Glochidion neilgherrense* showed least of 29% deterrency at 4% concentration, henceforth all the plants showed more than 50% of oviposition deterrency. The result of the present study is in agreement with the earlier findings on the ovipositional deterrent effect of different plants origin. Venkateswarlu *et al.* (1988) observed ovipositional deterrence of neem oil on *Spodoptera litura.* Ayyangar and Rao (1989) reported that the methanol and hexane of neem seed kernel extracts are not only larval repellents, but also ovipositional deterrent to the adult of *Spodoptera litura*. Raja *et al.* (2004) reported ovipositional deterrent activities of heaxane extract of *Aegle marmeles* and *Coleus aromaticus* and methanol extract of *Cyerus rotundus* and *Cyper aromaticus* at 5% concentration. Pure essential oil and individual compounds viz., geijerene and pregeijerene isolated form *Chloroxylon swietenia* showed oviposition deterrent activity against *Spodoptera litura.* The results of oviposition activity in the present study indicate that mosquitoes were acutely sensitive to chemical stimuli, with significant amounts of oviposition deterrent occurring in response to different concentrations of extract. Mosquitoes are known to select (or) reject their specific hosts and oviposition sites by sensing chemical signals that are detected by sensory receptors on the antennae (Davis and Bowen, 1994). The findings of present study are quite comparable with previous reports of Govindarajan *et al.* (2008a) have also observed that the leaf extract of *Azadirachta indica* with different solvents, viz., benzene, chloroform, ethyl acetate and methanol, had larvicidal activity, ovicidal activity and oviposition attractancy against *A. stephensi*.

In the present study, all concentrations of plant extracts used exhibited repellency activity against *Culex quinquefasciatus* females. The present study indicates that the methanol extraction of plants was more effective in exhibiting a repellency action against the mosquito tested compared to control. Many plant extracts and essential oils manifest repellency activity against different mosquito species. The present results are in accordance with results obtained by Sharma *et al*. (1993) using extracts from the seeds of *Azadirachta indica* against *Anopheles culicifacies* and *Culex quinquefasciatus*. Mansour *et al.*(1998) testing different extracts from *Nigella sativa* seeds against

C. pipens, Tuetun *et al*.(2004) using extracts of *Apium graveolens* seeds against *Aedes aegypti*, 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 of *Hyptis suaveolens* and *Rhododendon tomentosum.*

Morphological effects, almost all extracts of plant tested against all the instar larvae of *Culex quinquefasciatus* induced some morphological abnormalities in pupae and adults (pupal–adult intermediates) and incomplete or half – emerged adults were observed. The malformed pupae and adults were not able to develop normally and died. Similar observations were obtained with other plant extract against different mosquito species in earlier studes. Saxena *et al*. (1993) and Schmutterrer(1995) observed that *alkaloids* isolated for *Annona squamosa* induced morphological abnormalities such as larval–pupal intermediate and half – emerged adults in *Anopheles stephensi*; (Shalaby *et al*.(1998), Sharma *et al*.(2005) using peel oils of lemon, grapefruit and naval orange against *C. pipiens* observed adults with paralyzed legs which were not able to survive. Similarly, Abhaussain (1999) using *Calotro pisprocera* extracts against *C. pipiens an A. multicolor* observed morphological abnormalities in immature and adult stages of *Culex pipiens* when larvae were treated with the neem, *Azadirachta indica* extracts. In our observation almost all extracts against the larvae of *Culex quinquefasciatus*, induced some morphological abnormalities in pupae and adults. The malformed pupae were not able to develop normally and then died. Also, the present results showed that the percent and degree of malformation were concentrations dependent. In general it could be conclude that the plant extracts used in the present study act as larvicidal and possess growth and emergence inhibiting against the mosquito vector *Culex quinquefasciatus*. Furthermore, results of the present study may contribute to a reduction in the application of synthetic insecticides, which in turn increases the opportunity for natural control of various medically important pests by botanical pesticides. Further studies on the tested plants including mode of action, synergism with the biocides under field conditions are needed.

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