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RESEARCH ARTICLE

STUDIES ON BIOLOGICAL ACTIVITY OF ENDEMIC MEDICINAL PLANTS IN THE NIGIRI HILLS AGAINST THE FILARIAL VECTOR, *CULEX QUINQUEFASCIATUS* (SAY) (DIPTERA: CULICIDAE)

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ABSTRACT

In the present study the methanolic extract of *Artocarpus hirsutus* Lam., *Cinnamomum wightii* Meisner, *Diopyros paniculata* Dalz., *Garcinia gummi-gutta (L.) Robs. Garcinia indica* Choiss, *Michelia nilagrica* Zenk. *Rhododendron arboreum* (Zenk.) *Tagg.*) leaves were evaluated against *Culex quinquefasciatus*. *Artocarpus hirsutus, Cinnamomum wightii*, *Diopyros paniculata, Garcinia gummi-gutta*, *Garcinia indica*, *Michelia nilagrica* and *Rhododendron arboreum* leaves extract were tested for their biological, larvicidal, pupicidal, antiovipositional activity, repellency and deformities against *Culex quinquefasciatus.* The larval, pupal and adult mortality were increased significantly with increasing levels of plants leaves extract concentrations. The adult emergency also significantly affects by the treatment of all the plant leaves extract. The larval and pupal duration were extended by the treatment of all the plant leaves extract. Adult longevity and fecundity were greatly reduced after the treatment of plant extracts. At 4% the *Diopyros paniculata* leaves extract showed strong oviposional deterrency. The *Diopyros paniculata* leaves extract treatment significantly enhanced repellency activity.

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INTRODUCTION

Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. WHO has declared the mosquitoes as "public enemy number one". 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 Indian population. They act as a vector for most of the life threatening diseases like malaria, yellow fever, 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. *Culex quinquefasciatus*, is a vector of lymphatic filariasis which is a widely distributed tropical disease, and there are nearly 1,100 million people living in areas endemic for lymphatic filariasis and exposed to the risk of infection; there are 102 million cases of filariasis, either having patent microfilaraeimeia or chronic filarial disease (Michael *et al*.,1996).

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Wuchereria bancrofti accounts for approximately 90% of all filariasis cases in the world, followed by *Brugia malayi* and *Brugia timori*. India contributes about 40% of the total global burden of filariasis and accounts 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, Utter Pradesh, and west Bengal) contributed to about 95% of the total burden of filariasis.

To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organphosphate compounds. But this has not been very successful due to human, technical, operational, ecological, and economic factors. In recent years, use of many of the former synthetic insecticides in mosquito control programme has been limited. It is due to lack of novel insecticides, highly cost of synthetic insecticides, concern for environment sustainability, harmful effect on human health, and other non- target populations, their non biodegradable

nature, higher rate of biological magnification through ecosystem, and increasing insecticide resistance on a global scale (Russell *et al.,* 2009). Thus, the Environment protection Act in 1969 has framed a number of rules and regulation to check the application of chemical control agents in nature (Bhatt and Khana, 2009). It has prompted researchers to look for alternative approaches ranging from provision of or promoting the adoption of effective and transparent mosquito management strategies that focus on public education, monitoring and surveillance, source reduction and environment friendly least- toxic larval control. These factors have resulted in an urge to look for environment friendly, cost- effective, biodegradable and target specific insecticides against mosquito species. Considering these, the application of eco- friendly alternatives such as biological control of vectors as become the central focus of the control programme in lieu of the chemical insecticides.

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 concertedly on both behavioral and physiological processes. Thus there is very little chance of pests developing resistance to such substance. Identifying bio-insecticides that 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 may act as suitable alternative product to fight against mosquito borne diseases.

Botanicals are basically secondary metabolites that serve as a means of defense mechanism of the plants to withstand the continuous selection pressure from herbivore predators and other environment factors. Several groups of phytochemicals such as alkaloids, steroid, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities (Shaalan, *et al*., 2005). Insecticidal effects of plant extraction vary not only according to plant species, mosquito species, geographical verities and parts used, but also due to extraction methodology adapted and the polarity of the solvent used during extraction. 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 or from various parts like fruits, leaves, stems, barks, roots, *etc*., of large plants or trees. In all cases where the most toxic substances were concentrated upon, found and extracted for mosquito control.

Many plants produce secondary components that have insect growth inhibitory activity. Indeed, many plant extracts were studied for their 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 of seven plant extracts against the III instar larvae of *An. stephensi.* Personal protective measures, including repellents and larvicidals are widely used to prevent the transmission of arthropod borne diseases by minimizing the contact between humans and vectors. In contrast to vaccines and chemoprophylaxis as means of personal protection, repellents and larvicides are convenient, inexpensive, and offer advantages in protection against a wide range of vectors (WHO, 1995). They are also the primary means of mosquito-borne disease prevention available in areas where vector control is not practical (Gupta and Rutledge, 1994).

The majority of commercial repellent products contain the chemical DEET (diethyl-3-methylbenzamide, formerly known as diethyl-*m*-toluamide), which was first synthesized in 1954 (McCabe *et al.,*1954). More than 3,000 alkaloids have been identified in 4,000 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 and/or psychological results. The difference between a medicinal and a toxic effect of many alkaloids (or any drug) is often a matter of dosage (Levetin and McMahon 2003). Bioactive organic compounds produced by plants can act as repellent, oviposition or food deterrents, growth inhibitors, and toxins (Ezeonu *et al*., 2001; Carlini and Grossi-de-Sá, 2002). Thus, crude plant extracts have been screened as natural and biodegradable forms to control pests and vectors of infectious diseases (Omena *et al*., 2007). Sukumar *et al*., (1991) reviewed the bioactivity of 344 plant species against mosquitoes. They showed that some phytochemicals act as general toxicants to all life stages of mosquitoes, whereas others interfere with growth and reproduction or act on the olfactory receptors eliciting responses of attractancy or repellency.

A variety of secondary metabolites in the extracts obtained from different parts of a whole range of plants have been found to kill adult mosquitoes or reduce/inhibit feeding, egg laying, growth and development of mosquito larvae and pupae (Coria *et al*., 2008). The phytochemicals derived from plant resources can act as larvicides, adulticides, repellent and ovipositional attractants, having deterrent activities in different researchers and may be alternative sources of mosquito larval control agents (Kamaraj *et al*., 2009). In the present paper we have screened the endemic plants such as *Artocarpus hirsutus, Cinnamomum wightii* , *Diopyros paniculata, Garcinia gummi-gutta*, *Garcinia indica*, *Michelia nilagrica* and *Rhododendron arboreum* leaves extract on the larvicidal, pupicidal, adulticidal, larval, pupal and adult duration, reproductive activity and repellency of *C. quinquefasciatus*. The possible result of the present result would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive chemical compounds from indigenous endemic medicinal plant source.

MATERIALS AND METHODS

Preparation of Plant extracts

All the plant materials (*Artocarpus hirsutus* Lam*.,* *Cinnamomum wightii* Meisner, *Diopyros paniculata* Dalz*.,*

Garcinia gummi-gutta (L.) Robs. Garcinia indica Choiss, *Michelia nilagrica* Zenk. *Rhododendron arboreum* (Zenk.) *Tagg.*) were collected from the forest of Nilgiri hills. 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 (methanol) 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 with 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 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 replicate five times. Percentage mortality observed in the control was subtracted from that observed in the treatments (Abbot, 1925). The day from moulting of the larvae to pupation and to adulthood was noted. Fecundity was assessed by counting the number of eggs laid during the life span by control and experimental mosquitoes. The larvae and pupal duration of treated and control individuals were compared and developmental rates were determined.

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 was expose only to distilled water with methanol 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 give a choice between treated and control jars. During the tests, the groups of females were kept separate for 48h in cages measuring 25x25x30cm. After the eggs were counted the oviposition activity index (OAI) was calculated using the formula:

OAI = (Nc–Nt)/ (Nc+Nt) X 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 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 previously described (Murugan and Jeyabalan, 1999).

Statistical analysis

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

RESULTS

The total seven locally grown different plants were collected and the methanolic extracts of their leaves were tested for larvicidal, pupicidal, adulticidal, larval duration, pupal duration and adult duration, reproductive activity, repellency and deformities of *C. quinquefasciatus*. The assay of the investigated plant species were carried out using different concentration of methanol on *C. 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 at lower concentration was observed but exhibited higher activity as the concentration increase. The results of extracts of seven plants (*Artocarpus hirsutus, Cinnamomum wightii*, *Diopyros paniculata, Garcinia gummi-gutta*, *Garcinia indica*, *Michelia nilagrica* and *Rhododendron arboreum)* screened for their larvicidal activity against 1st, 2nd , 3rd and 4th instar larvae of *C. quinquefasciatus* were presented in Table 1.

All the seven plants with methanolic extracts showed promising larvicidal effects. As evidenced from the table, generally increased larval mortality was observed with increased concentration of the extracts tested against *C. quinquefasciatus*. Tables 1 shows the mortality after 24 hours caused by each plant extracts and the standard in chronological order. All the data showed that there was a highly significant difference between treatments and control. The mortality

progressively increased with increasing extract concentration. The plants were more effective at high concentrations, the toxic effect however increased with increase in the concentrations of the extracts.

and 2nd instar were more susceptible than the 3rd and 4th instar. The extracts caused harm to larvae and pupae during moulting, especially the time of metamorphosis. This strongly suggested that the action was a hormone mimic.

Table 1. Effect of methanolic extracts of plants on the larval mortality of *C. quinquefasciatus*

S. No	Treatment	Concentration (%)	1st Instars (%)	2nd Instars (%)	3rd Instars (%)	4th Instars (%)
	Control		00^{p}	00^{o}	00^{p}	00^{q}
1	*Artocarpus hirsutus*	1	18^{o}	15^{n}	11^{o}	7^{p}
		2	30^{mn}	36^{kl}	30^{kl}	25^{lm}
		4	41^{kl}	38^{jk}	34^{kl}	29^{lm}
2	*Rhododendron arboreum*	1	22^{no}	19^{n}	15^{o}	10^{p}
		2	34^{ln}	30^{lm}	26^{lm}	21^{no}
		4	44^{jk}	39^{jk}	35^{jk}	31^{kl}
	Michelia nilagrica	1	25^{no}	21^{mn}	17^{no}	12^{op}
3		2	37^{kl}	$32lm$	27^{kl}	22^{mn}
		4	49^{ij}	45^{ij}	40^{ij}	34^{jk}
4	*Garcinia gummi-gutta*	1	29^{mn}	24^{lm}	19^{mn}	14^{no}
		2	40^{kl}	36^{kl}	31^{kl}	27^{lm}
		4	53^{hi}	50^{hi}	46^{hi}	41^{ij}
5	*Garcinia indica*	1	59gh	55gh	50^{gh}	44^{hi}
		2	70^{ef}	66ef	61^{ef}	57^{fg}
		4	85^{bc}	80^{bc}	76^{bc}	71^{bc}
6	*Cinnamomum wightii*	1	64^{fg}	60^{fg}	56^{fg}	51^{gh}
		2	76^{de}	71^{de}	66^{de}	60^{df}
		4	91^{ab}	87^{ab}	81^{ab}	76^{ab}
7	*Diopyros paniculata*	1	69^{ef}	65^{ef}	60^{ef}	54^{fg}
		2	81^{cd}	67^{ef}	62^{ef}	58^{df}
		4	96^{a}	90^{a}	86^{a}	81^{a}

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

Table 2. Effect of Methanolic extracts of plants on the pupae and adults of *C. quinquefasciatus*

S. No.	Treatment	Concentration (%)	Pupal mortality (%)	Adult mortality (%)	Adult emergence (%)
	Control		00^{p}	00^{o}	00^{n}
1	*Artocarpus hirsutus*	1	19^{O}	16^{n}	85^{a}
		2	32^{lm}	28^{lm}	64^{ef}
		4	43^{jk}	37^{jk}	43^{hi}
2	*Rhododendron arboreum*	1	23^{no}	21^{mn}	84^{ab}
		2	36^{kl}	33^{kl}	62^{ef}
		4	45^{jk}	40^{jk}	41^{ij}
3	*Michelia nilagrica*	1	26^{mn}	22^{mn}	82^{ab}
		2	38^{kl}	36^{kl}	60^{ef}
		4	50^{ij}	48^{hi}	40^{ij}
4	*Garcinia gummi-gutta*	1	30^{mn}	23^{mn}	80^{ab}
		2	41^{kl}	38^{jk}	58^{ef}
		4	55^{hi}	51^{hi}	38^{ij}
5	*Garcinia indica*	1	51^{ij}	48^{hi}	65^{de}
		2	70^{ef}	67^{ef}	43^{hi}
		4	89^{bc}	82^{bc}	25^{kl}
6	*Cinnamomum wightii*	1	55^{hi}	51^{hi}	62ef
		2	78^{de}	72^{de}	41^{ij}
		4	96^{ab}	89^{ab}	23^{lm}
7	*Diopyros paniculata*	1	60^{gh}	61^{fg}	60^{ef}
		2	82^{cd}	68^{ef}	39^{ij}
		4	100^{a}	92^{a}	20^{m}

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

The overall results indicate that *Diopyros paniculata* extracts with methanol appeared highly effective against all the larval stages. According to the results obtained with methanolic extracts of plants acting on the early larval instar of *C. quinquefasciatus* than later stage like 3rd and 4th instars. Considering the different treated instar, the 4th instar showed generally less effect than the other stages. It was clear that, all concentrations eventually produced a high kill at a characteristic point in larval stage, whereas the lower dose treatments gave more dispersed actions. As in all cases, the 1st

However, from the experiments it is difficult to speculate on the actual mode of action of the each plant extract. At the high dose, death occurred most in the larval stage. The pupicidal and adulticidal activities of methanolic extracts of seven plants were summarized in Table 2. All the plants registered some amount of mortality in bioassays with methanolic extracts. The pupal and adult mortality were observed to increases as the concentrations of the test samples were raised. No mortality was recorded in respective control replicates. The adult emergence from pupae after permanent exposure to seven

plants with methanolic extracts showed in Table 2. Percentage adult emergence was very lower in methanolic extracts of *Diopyros paniculata* than other plants. Adult emergence inhibition against *C. quinquefasciatus* recorded at all the concentration of methanolic extracted plants treatment. Results of laboratory resting of the adult repellent activity and oviposition deterrent activity of solvent extracts of plants shown in Table 3.

treated bowels and the numbers of eggs laid were comparatively lower in treated bowels than those in untreated bowls irrespective of the total number of eggs laid both on treated or untreated bowls. At the highest concentrations of methanolic extracts of *Diopyros paniculata* significantly reduced egg laying of C. *quinquefasciatus*. Results revealed significantly difference between the no. of egg laid in treated and non treated bowl.

Table 3. Effect of methanolic extracts of plants on adult repellency and ovipositional deterrency of *C. quinquefasciatus*

S. No	Treatment	Concentration (%)	Adult repellency (%)	Ovipositional deterrency (%)
	Control		00^{n}	00^{n}
1	*Artocarpus hirsutus*	1	9^{m}	20^{m}
		2	19^{kl}	34^{kl}
		4	31^{ij}	52^{gh}
2	*Rhododendron arboreum*	1	10^{m}	22^{m}
		2	21^{kl}	36^{kl}
		4	36^{hi}	55^{gh}
3	*Michelia nilagrica*	1	13^{lm}	24^{m}
		2	24^{jk}	38^{jk}
		4	40^{gh}	58^{gh}
4	*Garcinia gummi-gutta*	1	15^{lm}	26^{m}
		2	26^{jk}	40^{ij}
		4	44^{fg}	62^{ef}
5	*Garcinia indica*	1	36^{hi}	42^{ij}
		2	47^{fg}	58^{gh}
		4	70^{bc}	85^{ab}
6	*Cinnamomum wightii*	1	40^{ef}	44^{ij}
		2	51^{ef}	60^{fg}
		4	75^{ab}	$89a^{b}$
7	*Diopyros paniculata*	1	44^{fg}	47^{ij}
		2	56^{de}	62^{ef}
		4	80^{a}	93^{a}

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

Table 4. Developmental duration of *C. quinquefasciatus* after the treatment of methanolic extracts of plants

S.No	Treatment	Concentration (%)	Total larval duration (days)			
			1^{st} Instars	2^{nd} Instars	3^{rd} Instars	4^{th} Instars
	Control		1.6^{i}	2.9^{i}	3.1^{h}	3.6^{h}
1	*Artocarpus hirsutus*	1	1.6^{i}	3.0^{i}	3.2^{h}	3.7^{h}
		2	1.9^{hi}	4.1^{gh}	4.3^{gh}	4.5^{gh}
		4	2.2^{gh}	5.2^{ef}	5.5^{ef}	5.8^{ef}
2	*Rhododendron arboreum*	1	1.9^{hi}	3.1^{i}	4.4^{gh}	4.7^{fg}
		2	2.2^{gh}	4.2^{gh}	4.6^{gh}	5.8^{ef}
		4	2.4^{gh}	5.3^{ef}	5.7^{ef}	6.1^{ef}
	Michelia nilagrica	1	2.3^{gh}	3.4^{i}	4.6^{gh}	5.9^{ef}
3		2	2.5^{gh}	4.3^{i}	4.8^{gh}	6.1^{ef}
		4	2.8^{fg}	5.5^{ef}	5.9^{d}	6.2^{de}
4	*Garcinia gummi-gutta*	1	2.6^{ef}	3.6^{hi}	4.8^{fg}	6.1^{ef}
		2	2.8^{fg}	4.5^{gh}	4.9^{fg}	6.2^{de}
		4	3.3^{ef}	5.8^{cd}	6.1^{cd}	6.3^{de}
5	*Garcinia indica*	1	4.3^{cd}	4.9^{ef}	6.1^{cd}	6.3^{de}
		2	4.7^{cd}	6.1^{cd}	6.3^{cd}	7.6^{ab}
		4	5.4^{ab}	7.5^{ab}	7.5^{ab}	7.9^{ab}
6	*Cinnamomum wightii*	1	4.5^{cd}	5.1^{ef}	$6.3c^{d}$	6.5^{cd}
		2	4.9^{bc}	6.3^{cd}	6.5^{cd}	7.8^{ab}
		4	5.7^{ab}	7.6^{ab}	7.7^{ab}	8.1^{ab}
7	*Diopyros paniculata*	1	4.7^{cd}	5.3^{ef}	6.5^{cd}	6.7^{cd}
		2	5.1^{bc}	6.5^{cd}	6.7^{cd}	8.1^{ab}
		4	6.1^{a}	7.8^{a}	7.9^{a}	8.4^{a}

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

The methonolic extracts of plants showed strong repellent activity against adult *C. quinquefasciatus* at 4% concentration. It was found that the effect of the various concentration of different treatment differ significantly. The data showed that exposure to plant extract inhibited overall oviposition in

The larvae took more time to develop to pupae in all the treatment compared to the control (Tables 4). At maximum concentration of 4% with methanolic extracts of plants gave prolonged the larval duration by all the instars compared with the control, the total development period was increased with

increasing concentration of treatments. Dose-response relationship was determined for plants applied to *C. quinquefasciatus*. The duration of larval instars and the total developmental time were prolonged.

in *C. quinquefasciatus*. The data also revealed gradual increase in pupal duration and decrease in adult longevity (Table 5). Effect of methanolic extract of plants on fecundity of *C. quinquefasciatus* is furnished in Table 6.

Table 5. Pupal and adult duration of *C. quinquefasciatus* after the treatment of methanolic extracts of plants

S.No	Treatment	Concentration (%)	Total Pupal duration (days)	Total adult duration (days)
	Control		3.0^{ij}	7.1^{a}
1	*Artocarpus hirsutus*	1	3.2^{i}	6.9^{a}
		2	4.4^{gh}	5.5^{de}
		4	5.3^{ef}	4.2^{gh}
2	*Rhododendron arboreum*	1	3.3^{i}	6.8^{ab}
		2	4.6^{gh}	5.4^{de}
		4	5.5^{ef}	4.1^{gh}
	Michelia nilagrica	1	3.4^{i}	6.6^{ab}
3		2	4.7^{gh}	5.2^{ef}
		4	5.8^{ef}	3.9^{hi}
4	*Garcinia gummi-gutta*	1	3.5^{i}	6.4^{ab}
		2	4.9^{gh}	5.0^{ef}
		4	6.1^{e}	3.6^{ij}
5	*Garcinia indica*	1	4.7^{gh}	5.5^{de}
		2	6.7^{cd}	4.0^{hi}
		4	7.9^{ab}	2.2^{lm}
6	*Cinnamomum wightii*	1	4.9^{gh}	5.4^{de}
		2	6.9^{cd}	3.9^{hi}
		4	8.2^{ab}	2.0^{m}
7	*Diopyros paniculata*	1	5.1^{g}	5.2^{ef}
		2	7.1^{c}	3.7^{ij}
		4	8.5^{a}	1.8^{m}

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

Table 6. Effect of methanolic extract of plants on fecundity and egg hatchability of *C. quinquefasciatus*

S. No	Treatment	Concentration (%)	Fecundity (no of eggs)	Egg hatchability (%)
	Control		248^{a}	99^{a}
1	*Artocarpus hirsutus*	1	228^{b}	93^{a}
		2	200^{cd}	72^{de}
		4	180^{ef}	$61f^{g}$
2	*Rhododendron arboreum*	1	220^{b}	90^{ab}
		2	198^{cd}	71^{de}
		4	183^{dc}	59^{gh}
	Michelia nilagrica	1	212^{b}	88^{ab}
3		2	195^{cd}	69^{ef}
		4	180^{ef}	57^{gh}
4	*Garcinia gummi-gutta*	1	206^{bc}	86^{ab}
		2	192^{cd}	67^{ef}
		4	177^{ef}	54^{gh}
5	*Garcinia indica*	1	181^{de}	66^{ef}
		2	170^{ef}	43^{ij}
		4	157^{g}	36^{kl}
6	*Cinnamomum wightii*	1	178^{ef}	65^{ef}
		2	167^{ef}	40^{jk}
		4	154^{g}	33^{kl}
7	*Diopyros paniculata*	1	175^{ef}	61^{fg}
		2	164^{fg}	38^{kl}
		4	150^{g}	30^{l}

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

In the present study, application of plant extracts greatly affected the growth of *C. quinquefasciatus*. During developmental metamorphosis, time taken for total larval and pupal developmental periods (in days), percent larval and pupal mortality, and adult emergence inhibition were recorded. Results revealed that treated individuals took prolonged larval and pupal period when compared to control in tested. Larval and pupal duration sgnificanly increased in treated individuals and total developmental period (larval and pupal development)

For determining the influence of methanolic extract of plants on the ovipositional pattern of *C. quinquefasciatus*, the mosquitoes were subjected to oviposition test. The total numbers of eggs laid in ovitraps containing any concentration of the methanolic extract of plants were always less than that in the control. Adult fecundity also was marked decreased by the plant extracts treatment. Among the total number of eggs laid 248 numbers was present in control medium when placed along with ovitraps with 1%, 2% and 4 % methanolic extract

of *Diopyros paniculata* in which appeared 175, 164 and 150 numbers of eggs respectively. The plants extracts drastically reduced the fecundity of the females, and only few adults survived.

viable and preferred alternative in the control of the mosquito species at the community level. Moreover, the plant should be locally available or easily cultivable at local level (Evans *et al.,* 1991).

Table 7. Effect of methanolic extracts of plants on larval-pupal intermediate of *C. quinquefasciatus*

S.No	Treatment	Concentration (%)	Larval-pupal intermediate (%)
	Control		00^{r}
1	*Artocarpus hirsutus*	1	4^{r}
		2	12^{pq}
		4	24^{jk}
2	*Rhododendron arboreum*	1	5^{r}
		2	14^{no}
		4	26^{jk}
3	*Michelia nilagrica*	1	6^{qr}
		2	15^{no}
		4	28^{ij}
4	*Garcinia gummi-gutta*	1	8^{pq}
		2	17^{no}
		4	29^{hi}
5	*Garcinia indica*	1	24^{jk}
		2	35^{fg}
		4	50bc
6	*Cinnamomum wightii*	1	26^{jk}
		2	37^{fg}
		4	53^{ab}
7	*Diopyros paniculata*	1	28^{jk}
		2	39^{fg}
		4	55^{a}

Effect of methanolic extract of *Artocarpus hirsutus, Cinnamomum wightii* , *Diopyros paniculata, Garcinia gummi-gutta*, *Garcinia indica*, *Michelia nilagrica* and *Rhododendron arboreum* on hatching of *C. quinquefasciatus* eggs freshly laid eggs obtained from the general stock of mosquitoes were tested for their hatching ability in relation to the different concentrations of methanolic extract of plants. Percent hatch of eggs placed in control medium was 99% where as in 1%, 2% and 4% concentrations of methanolic extracts of *Diopyros paniculata* was 61%, 38% and 30% respectively. The decrease in egg hatchability was found to be dose dependent.The result presented in Table 7 clearly indicated that lower concentration of the methanolic extracts of plants can effectively produced clear morphological growth disruption / malformations 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 shows several type of morphological malformations; deformed mouth brushed, melanised and scleoritized cuticles, light yellowish-albino coloured abdominal structures with a lack of peritrophic membrane outlines, compared to controls shows prominent mouth brushes and healthy cuticles. The pupae that survived through larval treatment showed a variety of malformation like complete demelanized pupa with straight abdomen, partly melanised pupa with extended abdomen, dwarf pupa with retarded abdomen, dechitinized pupa with distorted terminalia and pupa with defective genitalia.

DISCUSSION

Mosquito larvae control using larvicidal agents is a major component in the control of vector borne diseases. Thus, investigation onto plants as potential larvicides is considered as

Synthetic insecticides are today at the forefront of mosquito controlling agents. Nevertheless, controlling the mosquitoes has become complicated because 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 have been suggested as alternative for insect control because some are selective, biodegrade to nontoxic products, and have few effects on nontarget organisms and the environment (Pavela, 2007).

In view of residue problems in the environment and the development 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 suppress pest populations. Though several compounds of plant origin have been reported as insecticides-larvicides, there is a wide scope for the discovery of more effective plant products. Further research undoubtedly will lead the improved formulations with enhanced activity, which may eventually become environmentally acceptable and replace objectionable conventional insecticides for mosquito control. However there was a gradual overall mortality rate decreased as concentration decreased in the extracts of plants.

It was observed that there were significant differences between the low and higher concentrations of the extracts and higher mortality at higher concentration. This is consistent with the observation of Piyarat *et al.* (1974). Comparatively, *A.indica* compared favorably with *O. gratissimum* at higher concentration achieving 100% and 96% mortality after 24 hrs

of exposure at significant level $P \leq 0.05$ respectively, while *H. suaveolens* showed a high significant difference at $P \leq 0.01$. Earlier authors reported that the methanol leaf extracts of *Vitex negundo, Vitex trifolia, Vitex peduncularis* and *Vitex altissima* were used for larvicidal assay with LC_{50} value of 212.57, 41.41, 76.28 and 128.04 ppm, respectively, against the early fourth-instar larvae of *C. quinquefasciatus* (Kannathasan *et al*., 2007). In the present study, methanolic extracts of plants against *Culex quinquefasciatus* larvae showed higher mortality. In the present study, methanolic extracts was found to be effective larvicide and pupicidal agent. This extracts also interfered with the normal development and emergence of adult mosquitoes. Similar results were obtained in the studies on *A. squamosa* extracts against mosquito larvae (George and Vincent, 2005). The leaf extracts of *Cassia fistula* are known to have larvicidal and ovicidal activity on *Anopheles* and *Aedes* mosquitoes (Govindarajan, 2009).

Walmir *et al.* (2009) have reported that the isolation of bioactive compound ((+)-dicentrine) from *Ocotea velloziana* has larvicidal activity. The pesticide activity of crude extracts from a Lauraceous species, *Litsea salicifolia*, against *A. aegypti* has been reported by Phukan and Kalita (2005). Our results showed that crude extracts of plants have significant larvicidal and repellent activities against *C. quinquefasciatus* mosquitoes. The leaf extract of plants with methonal was tested for larvicidial and repellent activities against *C. quinquefasciatus.* The LC50 values were 18.56, 48.51, 49.57 and 50.32 ppm respectively (Mullai *et al*., 2008). Insecticidal and repellency effects of *A. digitata*leaves against *An. gambiae* have been first time studied in Nigeria. According to Denloye *et al.* (2006), pellets were made from pulverized waste wood, palm-kernel cake, dried "Kuka" (*A. digitata*) leaves and d-allethrin 90 EC and different pellets were prepared. All the pellets grades caused mortality of *An. gambiae* and *Musca domestica* when produced smoke. The hexane extract of *Andrographis paniculata* was more effective in exhibiting the repellent action against the mosquitoes as compared with *A. lineate* extract and the complete protection was observed for 150 min in hexane extract of *A. paniculata* at 500 ppm against *Cx. tritaeniorhynchus* bites (Elango *et al*., 2010).

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

The potential of these extract either having larvicidal or insecticidal activities has earlier been explored 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 (Umar *et al*., 2007; Abdelouabeb *et al*., 2009). The phytochemical screening results indicated that the leaves extracts of these plants were rich in alkaloids, flavonoids and tannins and 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 by other authors (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 Mulls 1999). This might be as a result of the plant constituent that have toxic effect on the mosquito larvae. This observation was further confirmed by Chakkaravarthy *et al*., (2011) who reported that biological activity of some plants extracts may be due to various compounds including flavonoids and alkaloid existing in the plants. 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 where 100% of mortality was found by methanolic extracts of plant leaves. In addition, almost all the plant extracts induced a reduction in the percentage of emerging adults from pupae produced from treated larvae. AI Dakhil and Morsy (1999) using the neem, *Azadirachta indica* extract against *C. pipiens* larvae, and Nathan *et al.* (2005) using methanol extracts of leaves and seeds of *Melia azedaracts* against *Anopheles stephensi* larvae.

The crude methanolic extracts from plants displayed larvicidal activity at varying levels. Previous investigations of *L. stellatus* indicated the crude extracts from 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 metabolised by insects in order to regulate their developmental processes (metamorphosis). Therefore, the compound when produces 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 a bio-insecticide. Therefore, the presence of this compound in plant extracts would make the extracts act as readily biodegradable environmental larvicides. The yield and activity of the most fractions determine the suitability of plant products for

mosquito control (Redwane *et al.,* 2002). Sukumar *et al.* (1991) suggested that the existence of variation in toxicities of phytochemical compounds on target species depending on the plant part from which they have been extracted. In addition, Jeyabalan *et al.* (2003) noted that other variation were due to responses of species and developmental stages of species to the specified extract, solvent of extraction, geographical origin of the plant, photosensitivity of compounds in the extract, effect on growth and reproduction, and other factors.

Inhibition of growth was detected with the extracts since all larvae grew to become pupae and subsequently adults. Earlier report (Bagavan, 2008) reveals that the ethyl acetate extract of *A. aspera* showed larvicidal activity against the early fourthinstar larvae of *Aedes aegypti* and *Cx. quinquefasciatus*. Either differences 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 findings of the present investigation were comparable with other ovicidal studies and revealed that the methanolic leaf extracts possesses ovicidal activity against the eggs of *C.quinqufasciatus.* In the case of ovicidal activity, exposure of freshly laid eggs was more effective than that of the older eggs (Miura *et al*., 1976). The methanolic extract treated eggs exhibited an allayed hatchability and this may be due to the action of phytochemicals present in the extract. The extract may inhibit the hatchability of the eggs by interfering with their chorion (Rajkumar *et al*., 2011). Eggs and egg shells treated with plant extracts become damaged probably due to endosmosis. After the initial phase of swelling, eggs become desiccated, followed by shrinkage and death of larvae trapped within (Arivoli and Samuel, 2011). It is also evident from the present study on exposure of *Ae. aegypti* eggs to the methanolic leaf extract of *S. campanulata*.

The treated eggs contained developed embryos the eclosion of the egg was incomplete (Miura *et al*., 1976). The growth of *C. 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 using different plant extracts against different mosquito species (Jeyabalan *et al.*, 2003; Shaalan *et al.,* 2005; Nathan *et al*., 2006; Sharma *et al.,* 2006 a &b). Morphological effects, Almost all extracts of plant parts tested against the 2nd instar larvae *of C. quinquefasciatus* induced some morphological abnormalities in pupae and adults (pupal- adult intermediates) and incompletely 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 studies. Shalaby *et al.* (1998) using peel oils of lemon, grapefruit and naval orange against *C. pipiens* observed adults with paralyzed legs which were not able to survive.

In the present study, almost all extracts, against the larvae of *C. 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. Similar observations were obtained by different plant extracts against different mosquito species as cited by Abahussain (1999) using *Calotropis procera* extracts against *C. pipiens* and *A. multicolor* observed morphological abnormalities among pupae. El–Bokl (2003) recorded varying degrees of morphogenic abnormalities in immatures and adult stages of *C. pipiens* when larvae were treated with the neem, *Azadirachta indica* extract.

These morphogenetic abnormalities are commonly caused by botanical extracts and are thought to result from a disturbance to growth regulating hormones (Saxena *et al*., 1993). In general, it could be concluded that almost the plant extracts used in the present study act as larvicidal, and possess growth and emergence inhibiting against the mosquito vector *C. quinquefasciatus*. Furthermore, the 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 condition are needed. Plant extracts may have an inhibiting influence on neurosecretory cells or may act directly on epidermal cells which are responsible for the production of enzymes for the tanning or cuticular oxidation process (Jeyabalan and Murugan, 1999). In all the treatments larvae became slowly inactive within 12h with degree of disturbance in behavior of the larvae as curling up, vigorous body movements, and discoloration which are the characteristic of neurotoxicity. A growing number of plant based products are very promising against mosquitoes and can be used as insecticides and/or repellents.

They offer a safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost. Most of the plant extracts used in this study has shown repellent effects against *Cx. quinquefasciatus* and may be useful for personal protection against the mosquitoes by individuals, thus minimizing the dependency on synthetic chemicals. These plant derivatives are probable sources of some biologically active compounds for mosquito control in the future. Since most of the plant based products are not as effective as synthetic insecticides and do not produce fast results, their use for mosquito control in a large scale programme under epidemic conditions may not be acceptable. However, the use of indigenous plant based products by individuals and communities can provide a prophylactic measure for protection against various mosquito borne diseases. There is a need for promoting the use of herbal products through community based vector control programmes.

In conclusion, plant extracts used in our study act as larvicidal, adulticidal, growth and emergence inhibiting, repellent and anti-feeding activities against the mosquito vector, *C. quinquefasciatus*. Furthermore, our results may lead to propose an alternative mean to naturally control various medically important pests in replacement to synthetic insecticides. These botanical pesticides are often active against specific target insects, less expensive, easily biodegradable in non- toxic products and potentially suitable for use in mosquito control program (Alkofahi *et al.,* 1989). Further studies on the

tested plants including mode of action, synergism with the biocides under field condition are needed.

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