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BIO-EFFICACY OF *LAVANDULA ANGUSTIFOLIA* (LAMIACEAE) AGAINST *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

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Abstract

Photochemical have proven that they are potential mosquito control agents and also alternative synthetic insecticides. Different concentration of plant leaf extracts of *Lavandula angustifolia* were tested for their activity against different larval and pupal stages of *Culex quinquefaciatus.* The larval and pupal mortality decreased after the treatment of plant extracts. The result obtained indicates better activity. Hence this plant extracts can be suitable alternative to synthetic insecticides. Similar observation is registered for 1st, 2nd, 3^{d} 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 petroleum ether and acetone. For instance, within the experimental level, the LC50 and LC90 values of *Lavandula arvensis* recorded 0.83 ppm and 3.07 ppm with acetone extract, 0.78 ppm and 2.80 ppm with petroleum ether extract, 0.70 ppm and 2.26 ppm with methanol leaf extract respectively. Hence it can be clearly seen that, methanol leaf extract exhibits highest mortality at low concentration (ppm), when compared to the plant and the solvents (petroleum ether and acetone).

Keywords: *Culex quinquefaciatus,* LC50, LC90 and *Lavandula angustifolia.*

Introduction

Diptera is one of the largest orders of insecta consisting of more than 85,000 species and it includes a large number of vectors. The predominant groups among these are mosquitoes which

are placed under the suborder Nematocera and family Culicidae. More than 3100 species of mosquitoes belonging to 34 genera have been recorded and arranged under three sub families namely, Anophelinae, Culicinae and Toxorhynchitinae (Knight and Stone, 1977). The genus *Culex* was first named and described by Linnaeus in 1735 and in 1823 Say named the species *Culex quinquefasciatus* (Dobrotworsky, 1965; Stone, 1956). Following this Meigen in 1818 described two more genera, *Aedes* and *Anopheles*. Mosquito transmit diseases like malaria, dengue, filariasis accounted for global mortality and morbidity with increased resistance to common insecticides. 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*., 2012; Ramar *et al*., 2013). Mosquitoes are found all over the world, except in Antartica. These two-winged insects belong to the order Diptera. Members of the genera *Anopheles*, *Culex* and *Aedes* are most commonly responsible for bites in humans. Mosquitoes spread disease to humans, domestic animals and wild life. Emergent mosquito-borne diseases, such as dengue fever and West Nile Virus (WNV), are recognized as an imminent health risk for people. *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. Lymphatic filariasis is a serious public health problem in India, constituting one third of the infected population in the world (WHO, 1997). Mosquito-borne diseases are endemic to India due to favourable ecological conditions for the vectors, their close contact with humans and their

reproductive biology. *Culex* lay "rafts" of eggs on still water in a variety of natural and man-made containers, including tree holes, ditches, sewage and septic tank water, catch basins (storm drains), non-chlorinated swimming and wading pools, decorative ponds, bird baths, flower pots, buckets, clogged gutters, abandoned tires and water-retaining junk and debris of all sorts. They cannot develop in running water and water that is present less than a week. Therefore, every effort should be made to prevent water from accumulating in containers or, at least, empty water out of them on a weekly basis. In rubber plantations, the rich organic content, stagnant water, low levels and protected conditions in the coconut shells used in rubber production favours intense breeding (Sumodan, 2003). Most of the insecticides available in the markets are synthetic chemical products which, apart from their prohibitively high costs, with their persistent applications have unintended implication including the production of resistant strains of mosquitoes, ecological imbalance and elimination of non-target organisms in the environment (Anyaele and Amusan, 2003). A total of 289 districts in India were surveyed for filiariasis up to 1995, out of which 257 were found to be endemic. In India a total of 553 million people are at risk of infection and there are approximately 21 million people with symptomatic filiariasis and 27 million microfilaria carriers. *Wuchereria bancrofti* is the national burden, widely distributed in 17 states and six union territories; B. malayi is restricted in distribution, with decreasing trend (ICMR Bulletin, 2003). An overview of the traditional endemic focus shows concentration of infection mainly around river basins and eastern–western coastal parts of India (Sabesan *et al*., 2000). 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 organophosphate 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, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health and other non-target populations, their non-bio-degradable nature, higher rate of biological magnification through ecosystem and increasing insecticide resistance on a global scale (Brown, 1986; Russel *et al*., 2009). Thus, the Environmental protection Act in 1969 has framed a number of rules and regulation to check the application of chemical control agents in nature (Bhatt and Khan, 2009). It has prompted researchers to look for alternative approaches ranging from provision to promoting the adoption of effective and

transparent mosquito management strategies that focus on public education, monitoring and surveillance, sources 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. 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; Yoganarashmhan, 2012). Due to the problem of pollution and vector resistance, safe plant products are being tested around the world as pest control agents (Ramaswamy *et al*., 2014; Bagavan *et al*., 2010). Medicinal plant have curative properties due to the presence of various complex chemical substances of different compositions, which are found as secondary plant metabolites in one or more parts of these plant (Prajapati and Purohit, 2003). 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. Botanicals are basically secondary metabolites that serve as a means of defense mechanism of the plant to with stand the continuous selection pressure from herbivore predator and other environmental factors. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plant 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 varieties and parts used, but also due to extraction methodology adapted and the polarity of the solvents used during extraction. 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 endemic plant of *Lavandula angustifolia* leaves extract on the larvicidal, activity 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 Method

Preparation of Plant extracts

The plant material *Lavandul*a *angustifolia* **(**Lamiaceae) field have been collected from the forest of Nilgri 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 methanol solvent 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 different solvents like Aceton, Petroleum ether and Methanol.

Mosquito culture

Mosquito larvae/eggs of *C. quinquefasciatus* have been collected in an around Nilgri hills. The mosquito colonies were maintained at $27 \pm 2\ ^{0}$C, 75-85% by the method of (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 different solvents 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).

Ovicidal assay

C. quinquefasciatus eggs were released in water. The test extracts were added in desired quantities and hatching were observed for one week. The eggs were then exposed to deoxygenated water and the numbers of hatching eggs were recorded. Percentage hatching was compared with the control in which only distilled water with different solvents 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).

Biting deterrency activity

The percentage protection in relation to dose method was used (WHO, 1996). Blood starved female *C. quinquefasciatus* (100 nos), 3-4 days old, was kept in a net cage (45x30x45 cm^2). The arm of the test person was cleaned with isopropanal. After air drying the arm, a 25 cm 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 solvent, distilled water with different 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 min. from 6 pm to 8 pm. The experiment was conduct five times. The percentage protection was calculated by using formula

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

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.

Results

Locally grown endemic plant was collected and the solvents extracts of their leaves were tested for larvicidal, activity of *Culex quinquefasciatus.* The assay of the investigated plant species were carried out using different concentration with three solvents (acetone, petroleum ether and methanol) on *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.

1. LC50 and LC90 values of acetone, petroleum ether and methanol leaf extract of plants against *Culex quinquefasciatus*

LC50 and C90 are unique values which represents the concentration required to kill 50% and 90% of the survival population in the study. In our present study the leaf extracts of *Lavandula angustifolia* with different solvents viz, acetone, petroleum ether and methanol was tested for larvicidal activity against *Culex quinquefasciatus* at variable and increasing concentration of 0.5%, 1%, 2%, 4% and 8% and the mortality were recorded for each instar stages and the LC50 and LC90 for (24hrs) values were computed, log transformed, assessed and represented in the form of Table (1 – 3) and figure 1 – 3 reported.

LC50 and LC90 values of the extracts with acetone Table (1) and Figure (1) , It has been observed that *Lavandula angustifolia* recorded with 0.68ppm;2.25ppm, 0.83ppm; 3.07ppm, 1.03ppm; 4.49ppm and 1.22ppm; 5.82ppm respectively with 1st instar, 2nd instar, 3rd instar and 4th instar larval stages . It has also been experimental 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 (ppm) of the extract to bring out the mortality.

The LC50 and LC90 values of petroleum ether leaf extract with *Lavandula angustifolia* were analyzed and represented in the Table (2) and Figure (2). It has been observed that *Lavandula angustifolia* recorded with 0.65ppm; 1.99ppm, 0.78ppm; 2.80ppm, 0.96.ppm; 3.86ppm and 1.13ppm; 5.10ppm respectively with 1st instar, 2nd instar, 3rd instar and 4th instars larval stages. The LC50 and LC90 values of petroleum ether leaf extract when compared to acetone leaf extract of the screened plant shows a clear interpretation that petroleum ether extract can bring considerable mortality of the larval forms at lower concentration(ppm), than acetone, which exhibits mortality at comparable high concentration(ppm).

The LC50 and LC90 values of Methanol leaf extract with *Lavandula angustifolia* were analyzed and represented in the Table (3) and Figure (3). It has been observed that *Lavandula angustifolia* recorded with 0.60ppm;1.56ppm, 0.70ppm; 2.26ppm, 0.85ppm; 3.29ppm and 0.98ppm; 4.13ppm respectively. Similar observation is registered for 1st 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 petroleum ether and acetone.

For instance, within the experimental level, the LC50 and LC90 values of *Lavandula arvensis* recorded 0.83ppm and 3.07ppm with acetone extract, 0.78ppm and 2.80 ppm with petroleum ether extract, 0.70ppm and 2.26 ppm with methanol leaf extract respectively. Similar observation is observed for the plant extract with the larval stages (Table 1-3) and (Figure 1 – 3). Thus it can be clearly seen that, methanol leaf extract exhibits highest mortality at low concentration (ppm), when compared to the plant and the solvents (petroleum ether and acetone).

LAVANDULA ANGUSTIFOLIA

Plate1: Experimental Plant used in our study

Table 1. LC50 and LC90 values of Acetone leaf extracts of *Lavandula angustifolia* against larvae of *Culex quinquefasciatus*

Mosquito Instar stages	LC50	LC90	95% Confidence limt				*χ2(df)*	Regression equation
			LC50		LC90			
			LCL	ULC	LCL	UCL		
1st Instar	0.68	2.25	0.56	0.79	1.90	2.83	3.52(3)	Y=-414+2.457X
2nd Instar	0.83	3.07	0.47	1.18	2.04	7.13	7.08(3)	Y=-184+2.252X
3rd Instar	1.03	4.49	0.54	1.56	2.71	14.76	8.92(3)	Y=-028+2.007X
4th Instar	1.22	5.82	0.72	1.80	3.50	17.80	7.26(3)	Y=-1.64+1.89X

LC50, LC90 = Lethal Concentration, LCL = Lower Confidence Limit, UCL = Upper confidence Limit, *χ2* = 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).

Figure 1. Larvicidal activity of acetone leaf extracts of *Lavandula angustifolia* against *Culex quinquefasciatus* expressed as LC50 and LC90.

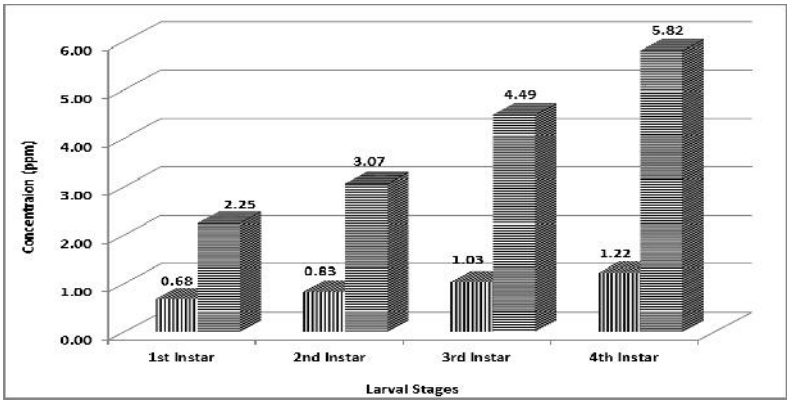


Table 2 . LC50 and LC90 values of Petroleum ether leaf extracts of *Lavandula angustifolia* against larvae of *Culex quinquefasciatus*

Mosquito Instar stages	LC50	LC90	95% Confidence limit				*χ2(df)*	Regression equation
			LC50		LC90			
			LCL	ULC	LCL	UCL		
1st Instar	0.65	1.99	0.54	0.75	1.68	2.47	1.88(3)	Y=-498+2.629X
2nd Instar	0.78	2.80	0.48	1.07	1.95	5.55	5.62(3)	Y=-250+2.308X
3rd Instar	0.96	3.86	0.45	1.51	2.30	15.01	11.05(3)	Y=-034+2.308X
4th Instar	1.13	5.10	0.65	1.69	3.06	16.54	8.29(3)	Y=-104+1.960X

LC50, LC90 = Lethal Concentration, LCL = Lower Confidence Limit, UCL = Upper confidence Limit, *χ2* = 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).

Figure 2. Larvicidal activity of petroleum ether leaf extracts of *Lavandula angustifolia* against *Culex quinquefasciatus* expressed as LC50 and LC90.

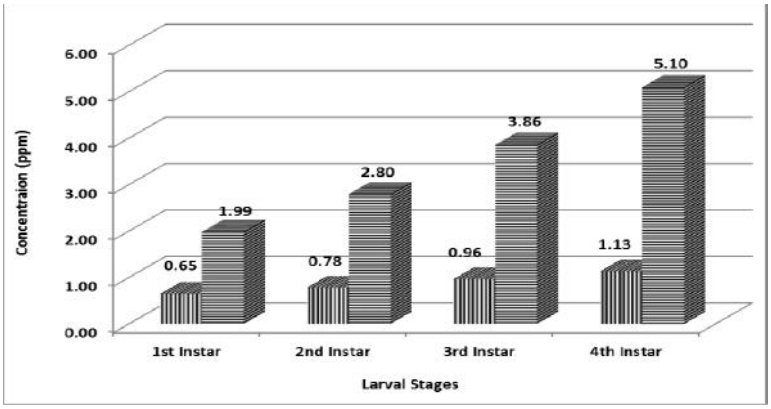
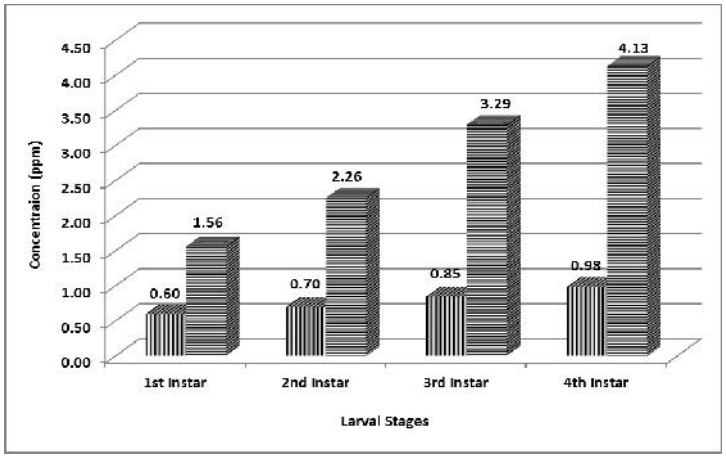


Table 3. LC50 and LC90 values of Methanol leaf extracts of *Lavandula angustifolia* against larvae of *Culex quinquefasciatus*

Mosquito Instar stages	LC50	LC90	95% Confidence limit				*χ2(df)*	Regression equation
			LC50		LC90			
			LCL	ULC	LCL	UCL		
1st Instar	0.60	1.56	0.50	0.68	1.32	1.99	0.26(3)	Y=-690+3.072X
2nd Instar	0.70	2.26	0.58	0.81	1.90	2.83	2.87(3)	Y=-395+2.508X
3rd Instar	0.85	3.29	0.45	1.24	2.11	8.84	8.10(3)	Y=-034+2.170X
4th Instar	0.98	4.13	0.50	1.50	2.62	16.26	9.53(3)	Y=-104+2.055X

LC50, LC90 = Lethal Concentration, LCL = Lower Confidence Limit, UCL = Upper confidence Limit, *χ2* = 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).

Figure 3 . Larvicidal activity of methanol leaf extracts of *Lavandula angustifolia* against *Culex quinquefasciatus* expressed as LC50 and LC90.



Discussion

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.,* 2006b). Our results showed high bioactivity of the different extracts from 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. 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 the methanol and acetone extraction. Many plant extracts and essential oils manifest repellency activity against different mosquito species.The present results are in accordance with results obtained by Sharma and Ansari (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,* Kim *et al.* (2002) using ethanol extracts of fruits from *Foeniculum vulgarea* against *Aedes aegypti* females, 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.* (2006b) using ethyl acetate extracts of *Hyptis suaveolens* and *Rhododendon tomentosum.*

In conclusion, plant extracts used in our study act as larvicidal, pupicidal, adulticidal, growth and emergence inhibition, repellent and biting deterrent activities against the mosquito vector *Culex 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; Swidan, 1994). Further studies on the tested plants including mode of action, synergism with the biocides under field condition are needed.

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