



## Mosquitocidal Activity of *Pogostemon nilagiricus* against the Filarial Mosquito, *Culex quinquefasciatus* Say

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### Abstract

Mosquitoes act as a vector for most of the life threatening diseases, like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, west Nile virus etc. *Culex quinquefasciatus* is a vector of lymphatic filariasis. Hence the emphasis on botanical insecticides which are more eco-friendly and effective. Plant extract from *Pogostemon nilagiricus* commonly available and growing in Nilgiri Hills (India) were tested for larvicidal activity against the 3<sup>rd</sup> instar larvae of *Culex quinquefasciatus* in laboratory conditions to evaluate the mosquitocidal activity of methanolic extracts of *Pogostemon nilagiricus* with different concentrations (1%, 2%, and 4%) of methanol extracts. The larvicidal, pupicidal and adulticidal activity were recorded after 24 hours under the laboratory conditions. 100% larval mortality was observed in 1<sup>st</sup> instar of *Culex quinquefasciatus* after the treatment of *Pogostemon nilagiricus* at 4% concentration whereas in 3<sup>rd</sup> and 4<sup>th</sup> instar larval mortality was 90% and 85% at 4% treatment respectively. The pupal mortality was 100% at 4% of plant extract treatment. Adult mortality was 98% after the treatment of *Pogostemon nilagiricus* leaves extract at 4%. But the adult emergence was drastically reduced after the treatment of plant extracts. The larval duration was greatly extended up to 42 days after the treatment of *Pogostemon nilagiricus* leaves extracts at 4% than other concentrations. Pupal duration also extended after the treatment of *Pogostemon nilagiricus* leaf extracts than control. But the adult duration was drastically reduced after the treatment of plant extracts. Fecundity and egg hatchability also reduced after the treatment of *Pogostemon nilagiricus* leaf extract. 96% ovipositional deterrence was observed after the treatment of plant extracts at 4%. Adult repellency was 88% after the treatment of plant extracts at 4%. Biting deterrence also increased after the treatment of plant extracts (1% < 2% < 4%). Larval pupal intermediate was very high after the treatment of *Pogostemon nilagiricus* leaves extracts.

**Keywords:** Mosquito, *Culex quinquefasciatus*, *Pogostemon nilagiricus*, Mortality, Biology, Reproduction, Repellency

### 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, in infecting over 700,000,000 people every year globally and 40,000,000 of the Indian population. About 3500 species of mosquitoes have been described worldwide. Relatively few of them are significant vectors of the life threatening diseases.

Mosquitoes spread disease to humans, domestic animals and wildlife. *Culex quinquefasciatus* is a vector of lymphatic filariasis which is a widely distributed tropical disease *wuchereria bancrofti* accounts for approximately 90% of all filariasis cases in the world, followed by *Bragia 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.

Thus, the Environment Protection Act (EPA) IN 1969 has framed a number of rules and regulation to check the application of chemicals control agent 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. 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. Phytochemicals are botanicals which are naturally occurring insecticides obtained from it control floral resources. Applications of phytochemicals in mosquito control were on use since the 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 to 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 then, the search for new bioactive compounds from the kingdom and an effort to determine its structure and commercial production has been initiated. At present phytochemicals make up to 1 percent of world's pesticide market (Isman, 1997).

In the present study screened the endemic plant *Pogostemon nilagiricus* leaves extract on the larvicidal, pupicidal, adulticidal, pupal and adult duration, reproductive activity, repellency and biting deterrence of *Culex quinquefasciatus*. The possible result of the present proposal 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

### Collection and preparation of plant extracts

Healthy leaves of *Pogostemon nilagiricus* 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, Udahgamandalam 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°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  $27 \pm 2$  °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<sub>50</sub> and LC<sub>90</sub> values and their 95% confidence limits were estimated for larval mortality by fitting a probit regression model to the observed relationship between percentage mortality of larvae and logarithmic concentration of the substance. Separate probit models were fitted for each extract (Finney, 1971)

The day from moulting of the larvae to pupation and to adulthood was noted. Fecundity was assessed by counting the number of eggs laid during the life span by control and experimental mosquitoes. The larvae and pupal duration of treated and control individuals were compared and developmental rates were determined.

### Adulticidal assay

*Culex quinquefasciatus* fresh adults were exposing to filter paper treated with different concentration of plant 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:

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

Where Nc is the number of eggs in the control  
Nt is the number of eggs in the treatment

### Ovicidal assay

*Culex quinquefasciatus* eggs were released in water. The test extracts were added in desired quantities and hatching were observed for one week. The eggs were then exposed to deoxygenated water and the numbers of hatching eggs were recorded. Percentage hatching was compared with the control in which only distilled water with 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<sup>2</sup>). The arm of the test person was cleaned with isopropanol. After air drying the arm, a 25 mc<sup>2</sup> 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:

Percentage protection=

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

### 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

Table 1 shows that methanolic extract of *Pogostemon nilagiricus* against *Culex quinquefasciatus*. It showed maximum larval mortality at 4 % concentration, when compared to the 2% and 4% concentration. It shows

maximum activity of pupal mortality, adult mortality and adult emergency. 100% pupal mortality was achieved at 4% concentration, when compared to the 2% and 1% concentration.

**Table 1. Toxic effect of methanolic extract of *Pogostemon nilagiricus* against *C. quinquefasciatus***

Treatment	Concentration (%)	Larval mortality (%)				Pupal mortality (%)	Adult mortality (%)	Adult emergency (%)
		1 <sup>st</sup> Instar	2 <sup>nd</sup> Instar	3 <sup>rd</sup> Instar	4 <sup>th</sup> Instar			
Control		00 <sup>d</sup>	00 <sup>d</sup>	00 <sup>d</sup>	00 <sup>d</sup>	00 <sup>d</sup>	00 <sup>d</sup>	00 <sup>d</sup>
<i>Pogostemon nilagiricus</i>	1	64 <sup>c</sup>	58 <sup>c</sup>	54 <sup>c</sup>	49 <sup>c</sup>	56 <sup>c</sup>	52 <sup>c</sup>	49 <sup>a</sup>
	2	83 <sup>b</sup>	80 <sup>b</sup>	75 <sup>b</sup>	70 <sup>b</sup>	90 <sup>b</sup>	86 <sup>b</sup>	27 <sup>b</sup>
	4	100 <sup>a</sup>	94 <sup>a</sup>	90 <sup>a</sup>	85 <sup>a</sup>	100 <sup>a</sup>	98 <sup>a</sup>	10 <sup>c</sup>

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

Table 2 shows that the methanolic extract of *Pogostemon nilagiricus* against *Culex quinquefasciatus*. It showed maximum activity of larval duration (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instars) at 4% concentration when compared to the 2% and 1% concentration. The pupal duration was extended

maximum on treatment of methanolic extract of *Pogostemon nilagiricus*. The adult duration was greatly reduces (7 days) after the treatment of *Pogostemon nilagiricus* at 4 %, when compared to the 2 % and 1% concentration ( 26 and 27 days).

**Table 2. Biological activity of *Pogostemon nilagiricus* leaf extracts against *C. quinquefasciatus***

Treatment	Concentration (%)	Total larval duration (days)				Total pupal duration (days)	Total adult duration (days)
		1 <sup>st</sup> Instar	2 <sup>nd</sup> Instar	3 <sup>rd</sup> Instar	4 <sup>th</sup> Instar		
Control	%	1.6 <sup>d</sup>	2.9 <sup>c</sup>	3.1 <sup>d</sup>	3.6 <sup>d</sup>	3.1 <sup>c</sup>	71 <sup>a</sup>
<i>Pogostemon nilagiricus</i>	1	2.6 <sup>c</sup>	3.1 <sup>c</sup>	4.2 <sup>c</sup>	6.3 <sup>c</sup>	3.8 <sup>c</sup>	37 <sup>b</sup>
	2	4.9 <sup>b</sup>	6.1 <sup>b</sup>	8.5 <sup>b</sup>	9.8 <sup>b</sup>	7.1 <sup>b</sup>	26 <sup>c</sup>
	4	8.7 <sup>a</sup>	9.9 <sup>a</sup>	12.2 <sup>a</sup>	13.2 <sup>a</sup>	9.7 <sup>a</sup>	7 <sup>d</sup>

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

Table 3 shows that the fecundity, egg hatchability and ovipositional deterreny of *Culex quinquefasciatus* after the treatment of methanolic extract of *Pogostemon nilagiricus*. It showed maximum activity

of fecundity, egg hatchability and 96% ovipositional deterreny at 4% concentration, when compared to the 2% and 1% concentration.

**Table 3. Reproductive activity of *C. quinquefasciatus* after the treatment of *Pogostemon nilagiricus***

Treatment	Concentration (%)	Fecundity (No. of eggs)	Eggs hatchability (%)	Ovipositional deterreny (%)
Control		248 <sup>a</sup>	100 <sup>a</sup>	00 <sup>d</sup>
<i>Pogostemon nilagiricus</i>	1	165 <sup>b</sup>	49 <sup>b</sup>	45 <sup>c</sup>
	2	150 <sup>c</sup>	25 <sup>c</sup>	69 <sup>b</sup>
	4	121 <sup>d</sup>	12 <sup>d</sup>	96 <sup>a</sup>

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

Table 4 shows that the methanolic extract of *Pogostemon nilagiricus* against *Culex quinquefasciatus*. It showed high value of adult

repellency. And 89% biting deterrency was achieved at 4 % concentration when compared to the 2% and 1% concentration of *Pogostemon nilagiricus*.

**Table 4. Adult repellency and biting deterrency activity of *Pogostemon nilagiricus* against *C. quinquefasciatus***

Treatment	Concentration (%)	Adult repellency (%)	Biting deterrency (%)
Control		00 <sup>d</sup>	00 <sup>d</sup>
<i>Pogostemon nilagiricus</i>	1	38 <sup>c</sup>	48 <sup>c</sup>
	2	61 <sup>b</sup>	76 <sup>b</sup>
	4	88 <sup>a</sup>	92 <sup>a</sup>

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

Table 5 shows that the larval-pupal intermediate after the treatment of methanolic extract of *Pogostemon nilagiricus* against *Culex quinquefasciatus*. It showed

maximum activity of larval-pupal intermediate at 4 % concentration when compared to the 2% and 1% concentration.

**Table 5. Larval-pupal intermediate of *C. quinquefasciatus* after the treatment of *Pogostemon nilagiricus* leaf extract**

Treatment	Concentration (%)	Larval pupal intermediate (%)
Control		00 <sup>d</sup>
<i>Pogostemon nilagiricus</i>	1	47 <sup>c</sup>
	2	63 <sup>b</sup>
	4	87 <sup>a</sup>

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

## Discussion

The result of this present study showed that methanolic leaf extract of *Pogostemon nilagiricus* was effective as mosquito larvicides. This is in line with results of the preliminary works done by Vatandoost and Vaziri (2004) using neem plant (*Azadirachta indica*) and Cavalcanti *et al.* (2004) using *O. gratissimum* and *H. suaveolens*.

However there was a gradual overall mortality rate decreased as concentration decreased in the extract of *Pogostemon nilagiricus*. 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  $P < 0.05$  respectively, while *H. suaveolens* showed a high significant difference at  $P < 0.01$ .

Toxicity of the tested plant extracts all the instars varied according to plant used and the extract concentration. The larval mortality present increased as extract concentration increased for plant extracts. The toxicity values of tested extracts from leaves of *Pogostemon nilagiricus* based on mortality values may be arranged in a decending order of concentration  $4 > 2 > 1 >$  extract. These results agree to some extent with the previously mentioned suggestions of Murugan and Jeyabalan (1999).

Extracts from the *Pogostemon nilagiricus* displayed larvicidal activity at varying levels. Since both insecticidal and antitumour activities are known to exert similar modes of cell action by blocking the cellular transport system Zafra Polo *et al.* (1996), the larvicidal efficacy of the *Uvaria* species were considered to be due to the C benzyl dihydrochalcones and flavanones metabolized by *Uvaria* species (Acheabach *et al.*, 1997).

A significant decrease in the percentage of larval pupation was found with *Pogostemon nilagiricus* extract tested. Moreover, the pupation was found to depend on the plant and the solvent used for extraction. The present study showed that plant extract had also a toxicity effect on pupae where 100% of mortality was found by methanol extract of plant leaves. In addition, almost all the plant extracts induced a reduction in the percentage of emerging adult from pupae produced from treatment larvae. The results of AI Dakhil and Morsy (1999) using the neem, *A. indica* extract against *C. pipiens* larvae, and Nathan *et al.* (2005) using methanol extracts of leaves seeds of *Melia azadaracts* against *Anopheles stephensi* larvae.

Regarding adulticidal effects, even though mortalities of mosquitoes exposed to extract as *Pogostemon nilagiricus* were significantly higher than the negative controls, they were also significantly lower than mortalities of *C. quinquefasciatus* exposed to a commercial insecticidal used as positive control. Result indicated that the methanol extract of plant leaves tested against the all instars larvae of *C. quinquefasciatus* had also a toxicity effect on adults with mortality. Similar results were obtained by Sharma *et al.* (2005) using peel oils of lemon, grapefruit and naval orange against *C. pipiens* larvae, Jeyabalan *et al.* (2003) using methanol extract of *Pelargonium citrosa* leaf against *Anopheles stephensis* and Nathan *et al.* (2005) using the neem, *A. indica* extract against *Anopheles stephensi*.

The result of the present study is agreement with the earlier findings on the ovipositional deterrent effect of *Pogostemon nilagiricus* 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 oviposition deterrent activities of hexane extract of *Aegle marmelos* and *Coleus aromaticus* and methanol extract of *Cyperus rotundus* and *C. aromaticus* at 5 % concentration. Anandan *et al.* (2010) reported ovipositional deterrent activity of *Hyptis suaveolens* and *Melochia corchorifolia* fractions isolated from ethyl acetate extract against gravid moths of *Spodoptera litura*.

Our results showed that crude extracts of plant have significant larvicidal and repellent activities against *C. quinquefasciatus* mosquitoes. The leaf extract of plant with methanol was tested for larvicidal and repellent

activities against *C. quinquefasciatus* (Mullai *et al.*, 2008). Insecticidal and repellency effects of *A. digitata* leaves against *An. Gambiae* have been first time studied in Nigeria (Denloye *et al.*, 2006).

The compounds include saponin, tannin, alkaloid and flavonoid which might have contributed to the larvicidal potency displayed in this study. This agreed favourably with the report of Isman (1997) who reported that natural defense of plant against insects consist almost mixtures of closely related compounds rather than a single toxicant. The results from this present study also confirmed the previous works of using plant as insecticides. Fernando (2005) reported that most plant are known to possess chemicals substances like terpenoides, saponins, tannins flavonoids and alkaloids among others which are found to have reasonable efficacy against a range of mosquito species.

The plant tested in the present study is known to be non toxic to vertebrates. Moreover, it has been clearly proved that crude or and highly efficient for the control of mosquitoes rather than the purified compounds or extracts (Cavalcanti *et al.*, 2004; Jaenson *et al.*, 2006). Our result showed high bioactivity of the different extracts from the plant which is widely common in India. All concentrations of plant extract used exhibited repellency activity against *C. quinquefasciatus* female. 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 control. The present studys are in accordance which results obtained by Sharma and Ansari (1995) using extracts from the seeds of *A. indica* against *Anophelos culicifacies*.

The repellent assays showed promising results as the methanolic extracts of *Pogostemon nilagiricus* have strong repellency effects against *C. quinquefasciatus* and have potential as products for personal production against the mosquitoes. The extract of *Pogostemon nilagiricus* showed good repellence and were as effective as DEET against the filarial vector *C. quinquefasciatus*. This could be attributed to the fact that it belongs to the family capparaceae which has a high concentration of chemicals such as stachydrine and 3-hydroxy-4-methoxy-3-methyl-oxindole (Ravan *et al.*, 1999). The methanolic extract displayed greater acitivity because polar solvents extract more volatiles have been reported to have a strong repellency and mortality activity (Choochote *et al.*, 2004).

The methanol extract of *Pogostemon nilagiricus* showed a greater high knockdown after 60 min exposure. This could be attributed to the fact that it belongs to the family lamiaceae which has a high concentration of chemicals such as rosmarinic acid and other derivatives of caffeic acid (Raven *et al.*, 1999) and diterpenoids (Van wyk and Gericke, 2003) that have shown repellent activity against mosquitoes (Tunon *et al.*, 1994).


## Conclusion

Present study concluded that the plant extract used in our study act as larvicidal, adulticidal, growth, emergence inhibiting, repellent and anti-feeding activities against the mosquito vector, *C. quinquefasciatus*. Furthermore our results may lead 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.

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