



**LARVICIDAL ACTIVITY OF METHANOL EXTRACT OF *NERIUM OLEANDER* AND *TRIGONELLA FOENUM* AGAINST *CULEX* MOSQUITO LARVAE, ALONG WITH A SURVEY OF ENTIRE VELLORE DISTRICT FOR MOSQUITO**

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**ABSTRACT**

The aim of the study is to test the Larvicidal activity of methanol extract of *Nerium Oleander* and *Trigonella Foenum*, individually and in combination. Then to compare the result with the result obtained from the previous study with the aqueous extract of the same plants. A survey within Vellore district was done to check for the distribution and population of genera present. The study was done only on *Culex* genera, due to its vast availability, due to the level of tolerance it demonstrated against the aqueous extract and also due to level of threat this genera posses for the public. The results obtained from Larvicidal assay with the methanol extract is considerably higher than the aqueous extract, though with a disappointment that the combination of *Nerium oleander* and *Trigonella foenum* methanol extract did not provide a drastic scale up, as it did with the combination of aqueous extract.

**KEYWORDS** :*Nerium oleander*, *Trigonella foenum*, *Culex*, Larvicidal Assay, Vellore, Methanol extract.



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## INTRODUCTION

Mosquitoes transmit disease to more than 700, 000, 000 people annually and are responsible for the death of 1 of every 17 people currently alive<sup>1</sup>. Other than being the major protozoan carrier for malaria, mosquito also transmit the arboviruses responsible for yellow fever, dengue haemorrhagic fever, epidemic polyarthritis and several forms of encephalitis, some of which are found in India<sup>1</sup>. Dengue has been the recent epidemic breakout leading to deaths of more than 1000 people. Dengue infection ranges from asymptomatic to life threatening dengue, haemorrhagic fever/ dengue shock syndrome. Each year 50,000 patients are hospitalized with severe cases worldwide, it has been estimated that 2.5 billion people are at risk of infection<sup>2</sup>. Although many mosquito genera are dangerous and transmit several diseases, *Culex* is the most difficult one to eradicate and also to control<sup>3</sup>. *Culex*, widely distributed mosquito in India, acts as a vector for the cause of many important diseases such as avian malaria, Japanese encephalitis, West Nile virus, bancroftian filariasis (*Wuchereria bancrofti*). In India, 23 million cases of filariasis, 31 million microfilaraemics and about 473 million individuals are potentially at risk of getting infected by *Culex*<sup>3</sup>. The persistent uses of synthetic insecticides are causing biomagnifications, environmental pollution, and development of genetic resistance to mosquitoes and toxicity to non-target organisms. Continuous efforts to eradicate such vectors have resulted in vain. There is an immense need to develop target specific insecticides that are degradable and safe to the environment<sup>3</sup>. In this particular study methanol extracts of fenugreek (*Trigonella foenum*) and Oleander (*Nerium oleander*) are considered, because the previous study of the aqueous extracts of these plants proved the presence of larvicidal activity<sup>4</sup>. These plants constitute a rich source of bioactive compounds that are biodegradable and also contribute the effective control of the mosquito larvae.

## MATERIALS AND METHODS

### (i) Survey within Vellore District

Scheduled visits for each part of the Vellore district was done, so as to cover the entire area. The weather factors were taken into account and so the visits are done only during clear and warm climates, making sure the maximum larvae availability. These visits were done thrice so as to obtain an average number of sites each mosquito genera were present. The larvae samples from each site were collected in different containers and were brought to Research Lab in VIT University, Vellore, TN, India. Where the larvae were studied microscopically and the different genera were noted down, based on the guidelines of "Identification of the U.S mosquito larvae – manual"<sup>5</sup>.

### (ii) Larvae Collection

Several sites within the Vellore district identified during the survey, acted as larvae collection point for collecting *Culex* mosquito larvae, as the availability of this genera mosquito larvae was huge; approximately ~200 larvae/collection. These collection points include abandoned wells, small dirty pond and large unused land areas filled with rain water and sewage. Other local points of collection were also used, so as to get a total of ~300 larvae/ per collection. The collection was done at 3 days interval. The collection points were never depleted *i.e.*, always a minimum of 10% larvae was left behind, making sure not to deplete or reduce the availability of the larvae.

### (iii) Plant Sample

Fenugreek leaves (*Trigonella foenum*) and *Nerium oleander* leaves were purchased in Vellore district, Tamil Nadu, India. Care was taken to make sure that the sample leaves obtained are fresh, un-infected, not damaged or bitten by animals or insect and also that it was well maintained.

### (iv) Methanolic Extract

The fresh leaves are washed with distilled water and were shade dried to retain their biological activity. The dry plant leaves are finely ground with mortar and pestle. The

ground leaf is then soaked in methanol for 3 days. Then the methanol is filtered using Whatmann no.1 filter paper. The filtrate is kept in an open Petri dish for evaporation. After the alcohol was evaporated, the compound dried at the bottom was scrapped off. One gram of the scrapped powder was dissolved in 1 litre of distilled water to make 1000ppm concentration. It was then diluted to 100ppm, 150ppm and 200ppm concentration.

**(v) Larvicidal Assay**

Larvicidal assay was performed based on the guidelines of WHO, 1981<sup>6</sup>. The larvae were released into different concentration of test extract and were observed for 24, 48 & 72 hours, the number of dead larvae were counted at these time intervals, Control tests were also done alongside with the sample

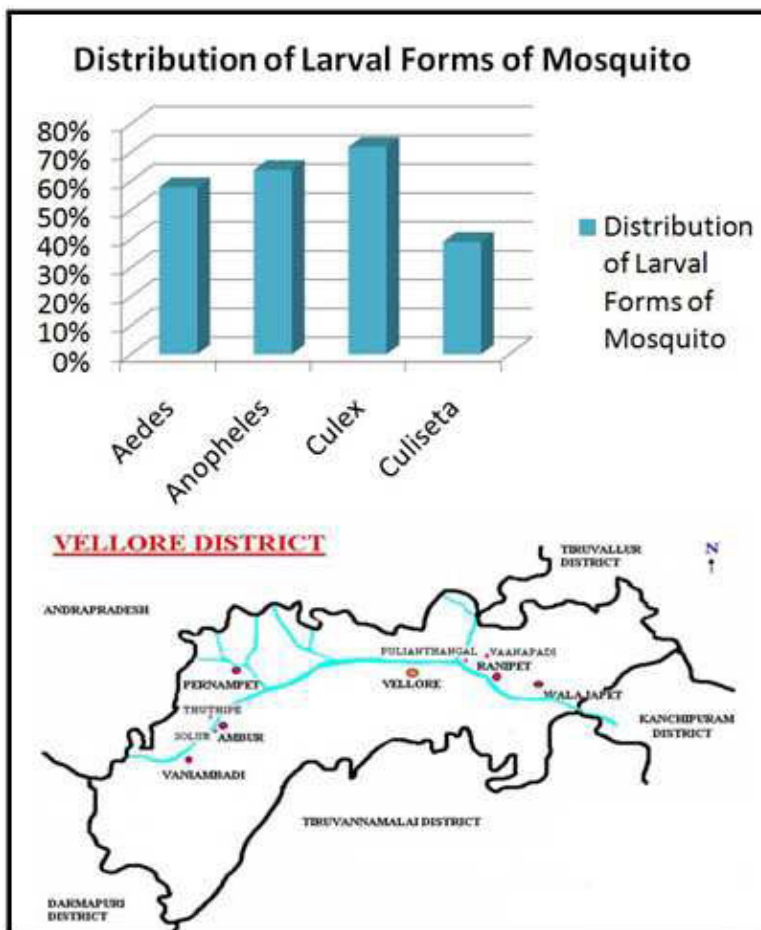
assay in reference with previous Larvicidal assay reports<sup>7, 8, 9, 10, 11 & 12</sup>.

**RESULTS**

**1. Survey for Mosquito Distribution throughout Vellore District.**

Survey revealed that all four genera of mosquitoes (*Culex*, *Aedes*, *Anopheles* and *Culiseta*) found in the previous study<sup>4</sup> were present throughout the district. The distribution pie chart given in Figure 1, was plotted based on the number of sites each genera were spotted. This survey covered the main areas of Vellore District such as; Vellore, Vaniambadi, Ambur, Pernampet, Ranipet, Walajapet, Solur, Pulianthangal, Vaanapadi, Thuthipe and the rural and interior sites, as shown in Figure.1.

**Figure 1**  
**Distribution of Larval forms of Mosquitoes throughout Vellore District**



The *Culex* scored the maximum number of spotted sites, following this were *Anopheles*, *Aedes* and *Culiseta* in the respective order. Statistically 72% of the entire surveyed areas had *Culex*, 64% had *Anopheles*, 58% had *Aedes* and 39% had *Culiseta*. Although *Culex*, *Anopheles* and *Aedes* genera were available in large amount, *Culex* particularly had impressive amount of larvae population, dominating the others. *Culiseta* had very few numbers of larvae at each site. The larvae collection point identified during the survey were the sites at which *Culex* larvae existed in enormous amounts and dominated the other genera larvae in that area.

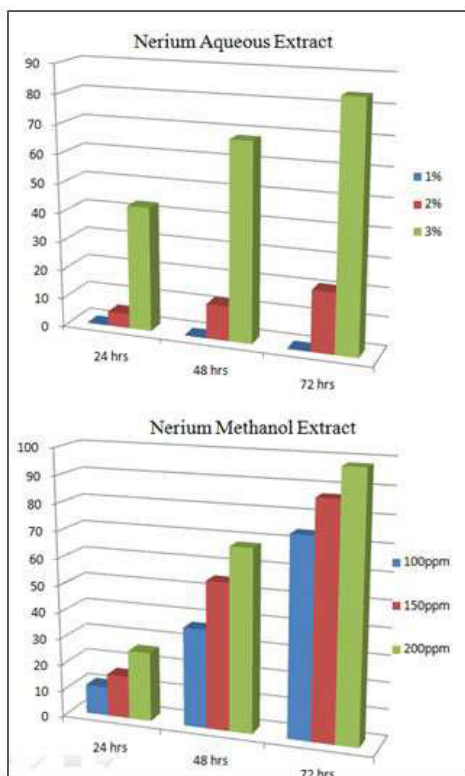
## 2. Larvicidal Assay of Methanol Extract

Results obtained from larvicidal assay, proved the presence of increased concentration of larvicidal compounds in methanol extract when compared to aqueous extract. As given in the Graph 1 and 2, the methanolic extract of *Nerium oleander* and *Trigonella foenum* was twice as effective as the aqueous extract,

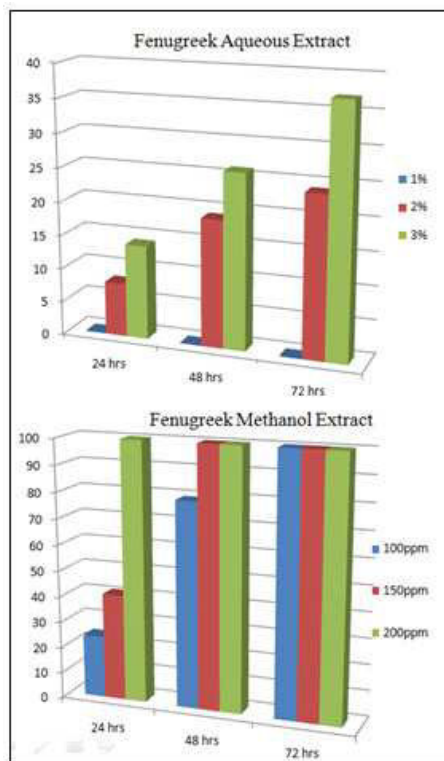
even at the 100ppm concentration, proving the concentrated larvicidal agents present in the methanolic extract. *Nerium oleander* did not show any larvae mortality in 1% aqueous extract, whereas 100ppm of *Nerium oleander* had 74% mortality in 72hours. *Trigonella foenum* also demonstrated 0% larvae mortality in 1% aqueous extract, whereas 100ppm of *Trigonella foenum* had 100% mortality in 72hours, demonstrating increased larvicidal activity, as shown in Table.1.

Following this, Graph 3, shows the comparison of Combination effect of *Nerium oleander* and *Trigonella foenum*. The combination of the aqueous extracts, drastically increased the larvicidal activity, where as the combination of methanolic extract, did not increase the activity. Instead, the maximum efficiency of *Trigonella foenum*, which demonstrated 100% mortality at 100ppm in 72hours and at 200ppm in 24hours, was reduced during the combination, as shown in Table.1.

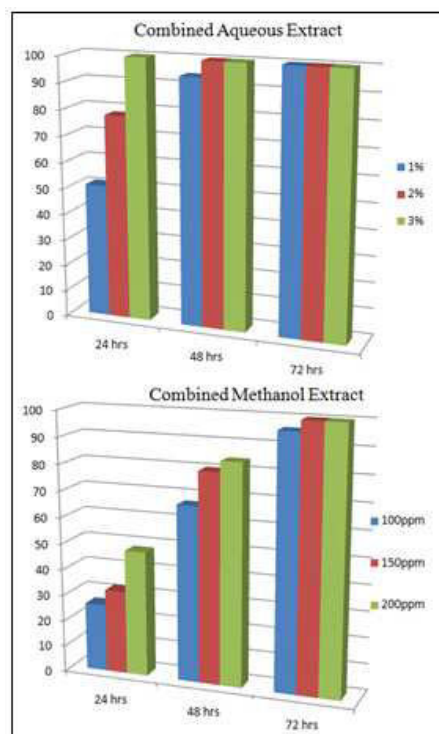
**Graph 1**  
**Comparison of Larvicidal activity of Aqueous Extract and Methanol Extract of Oleander (*Nerium oleander*), against *Culex* larvae**



**Graph 2**  
**Comparison of Larvicidal activity of Aqueous Extract and Methanol Extract of *Trigonella foenum* (Fenugreek), against *Culex* larvae**



**Graph 3**  
**Comparison of Larvicidal activity of Combined Aqueous extract and Combined Methanol extract (Combination of *Nerium oleander* and *Trigonella foenum*)**



**Table 1**  
**Larvicidal assay of Methanol Extract of Nerium oleander and Trigonella foenum Individually and in Combination against Culex Mosquito larvae**

Extracts	Concentration	Period of exposure		
		24hrs	48hrs	72hrs
Nerium	100ppm	11 ± 1	37±1	74±2
	150ppm	16± 3	55 ± 2	87±2
	200ppm	26 ± 1	68± 3	98±1
Fenugreek	100ppm	23±1	79±3	100±0
	150ppm	41±1	100±0	100±0
	200ppm	100±0	100±0	100±0
Combination	100ppm	20±2	42±1	61±2
	150ppm	32±3	50±2	74±3
	200ppm	39±2	58±2	86±1

## DISCUSSION

*Culex* mosquito seems to dominate the other genera of mosquitoes in regards with population. Among the three genera tested against the aqueous extract in the previous study, *Anopheles* showed high resistance, while *Aedes* was highly susceptible and *Culex* had a good level of tolerance<sup>4</sup>, making it a good choice for further study with concentrated sample like methanolic extract. Also the demanding need for controlling *Culex* mosquito made this study focus on *Culex* genera. We expected the larvicidal activity of the Methanol extract to be higher than the aqueous extract, which was basically the hypothesis of this work. The reduced larvicidal activity of the Combined methanol extract was unexpected and also disappointing. As the combined aqueous extract of the same plants had increased activity<sup>4</sup>, we expected similar results with the methanol combination. We propose this is because of the dilution of methanol extract in the combination and also due to the absence of other agents that were potentially present in the aqueous extract; Theoretically 100ppm of the methanol extract combination had 50ppm of *Trigonella foenum* and 50ppm of *Nerium oleander*, this dilution reduced the maximum activity of the

*Trigonella foenum*; also the potential ingredients that was present in aqueous extract which might have acted as a strong larvicidal agents during the combination. This hypothesis has to be studied further in the following research.

## CONCLUSION

This study has concluded that methanolic extract of *Nerium oleander* and *Trigonella foenum* possess larvicidal agents, which can be used as a biological remedy to control the mosquito population in the locality, especially the *Culex* mosquito which poses great threat of transmitting lethal diseases. The methanolic extract of these plants has higher larvicidal activity than the aqueous extract of the same plants. Also, combined methanol extract of *Nerium oleander* and *Trigonella foenum* had reduced activity than the individual plant extracts, for which the theoretical reason would be the dilution factor in the combination process. So, this study concludes that, the methanol extract of *Trigonella foenum* and *Nerium oleander* individually can potentially be used as an alternative to chemical insecticides.

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