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Larvicidal Activity of *Trigonella foenum* and *Nerium oleander* Leaves Against Mosquito Larvae Found in Vellore City, India

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Abstract: The aim of this study is to evaluate larvicidal activityof *Trigonella foenum* and *Nerium oleander* against mosquito and to test their activity in combination with each other. Mosquito, the primary vector for malaria, dengue and other severe infectious diseases are the major problem in Vellore city. Survey on the prevalence of mosquitoes present in Vellore city and the larvicidal activity of *Trigonella foenum* and *Nerium oleander* leaf extracts on the different mosquito larvae were studied. Further we studied the larvicidal effect of the combination of both the plant extracts. The results showed that, there are four genera of mosquitoes present in Vellore city and the leaf extract of both the above plants contains larvicidal activity for the most available genera in the survey, and the combination of the extracts, increased the activity considerably to a high percent.

Key words: Larvicidal activity, *Nerium oleander*, *Trigonella foenum,* Vellore

INTRODUCTION

The historical Vellore city is habitat of a huge biodiversity, in which mosquitoes are present in large amount. The vast number of mosquitoes present naturally causes the civilians to use mosquito coils and liquidators, which release Chlorofluorocarbon in a considerable amount that depletes the ozone, which is very harmful for the earth and human's future. These mosquitoes are vastly present in the developing countries, where not much importance is given to the sanitation. Rain water and sewage can easily get stagnant in the roads and in the open spaces, these water stagnant and open sewage passages acts as a very good habitat for the mosquitoes to breed, which seriously concerns the civilian's day to day life.

The mosquitoes are the important and major vectors for the severe and highly infectious diseases to humans and animals. Anopheles is an important vector for the transmission of malaria (Gutie *et al*., 2008; Manguin *et al*., 2008), Aedes is known for the transmission of Yellow fever (Fonteuillel *et al*., 1997) and Culex is known for transmission of filariasis in human and lumbar paralysis in cattle (Kwong-Chung *et al*., 2004; Merelo-lobl *et al*., 2003). Other works are being conducted to determine the diseases transmitted by mosquitoes, like Western Equine Encephalitis and Rift Valley.

Fever virus, by Culex species, (Gabriela *et al*., 1990; Moutailler *et al*., 2007). By acting as a primary vector for the pathogens that cause severe infections, elimination of mosquitoes is a must in the developing countries. Since the use of insecticidal sprays and other chemicals for killing the adult mosquitoes will affect the natural environmental conditions. Researches' are going on for the environmental friendly method to eliminate the mosquitoes, by use of biological compounds.

One such method is the use of plant extracts to kill the mosquito larvae in the water bodies, since killing of the larvae will terminate the life cycle (Fig. 1) and thus preventing the further multiplication of the mosquitoes. On effective application of this strategy the larvaes will be killed and in a shorter period, the natural aging process will also kill the adult mosquitoes present. Many researches has been done in this method with different type of extracts from various plant and tree parts, shrubs, fruits, etc., Toxicity tolerance towards specific chemicals and leaf extract has been tested on mosquito larvae. (Abdul *et al.,* 2008; Bishnu and Zeev, 2005; Das *et al*., 2007; Badruddoza, 2008; Davida *et al*., 2006; Hanem and Abdel-Salam, 2007; Nandita *et al.,* 2008).

The Fenugreek (*Trigonella foenum*) is an annual herb of Leguminosae. It is mostly cultivated in India, Pakistan, Egypt and Middle East countries. Its seeds are being used as spice and leaf as vegetable (Udayasekhara *et al.,* 1996). This plant has proved to contain many medicinal values such as anti-diabetic activity in animal models and proved to be beneficial in human subjects with non-insulin dependent diabetes, have anti-oxidant potential (Nooman *et al*., 2008) and hypocholoesterolemic activity of its seeds has been observed in animal and human subjects (Ebubekir *et al.,* 2005; Nazar *et al.,* 2007). It is also having valuable components like galactomannan (Ramesh *et al*., 2001), which is not widely exploited. The proteins from this plant are observed to be antigenic in nature, in

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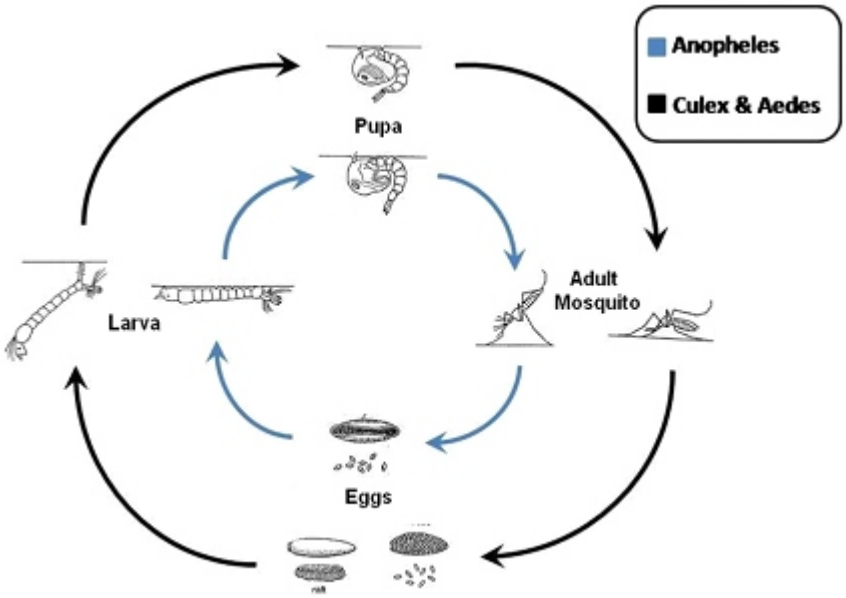

Fig 1: The Schematic representation of the life cycle of the *Anopheles, Aedes* and *Culex*. The blue indicating the *Anopheles* mosquito and the black indicating *Aedes* and *Culex*

Fig 2: The Picture of Nerium oleander, a common ornamental plant

some cases (Christiane *et al.,* 2009), and presence of valuable steroids like saponins are also reported in its seeds (Petit *et al.,* 1995).

The *Nerium oleander* (Fig. 2) is an evergreen flowering shrub of Dogbane family. It grows in tropical and subtropical regions. In history this plant has been used in medicine. It is popularly used as an ornamental plant, for its evergreen nature. This plant is proved to be toxic to human and animals (Al-Yahya *et al.,* 2000; Langford and Boor, 1996), but it is also proved to contain medicinal value like antibacterial activity (Mostaqul *et al.,* 1999), Anti-inflammatory and Antinociceptive activity (Erdemoglu *et al.,* 2003), and with these considerations, this plant is now being studied for its uses in medicine, yet with caution. Valuable compounds like cardiac steroid, arabinogalactan and Cardenolides are reported to be present in this plant (Mostaqul *et al.,* 1999; Qun and Ji-nian, 2001; Sabira *et al.,* 1999).

The objective of the study includes the survey of mosquito prevalence in the Vellore city, Tamilnadu, India. Survey and sampling of *Trigonella foenum* and *Nerium oleander* plants. Aqueous extraction of these plants and checking the Larvicidal activity against mosquito larvae.

MATERIALS AND METHODS

Mosquito survey: The different types of mosquito larvae are collected from various parts of the Vellore city and are brought to the research lab, VIT University, Vellore, India in a vessel with the water sample from the survey point. Then each larva were studied under microscope in 10x and 40x and are classified into different genera, based on "Identification of the U.S mosquito larvae - manual" (3), and the different types found in the city are recorded.

Larvae collection: The survey location includes- stagnant water near roads and streets, open sewage paths, unused wells, waters in the garbage dump and all other possible contaminated water of the Vellore city, which covers from Katpadi to Bagayam. The study started during the early February 2009 and was carried out till Late July 2009.

Huge numbers of larvae were available in the wells; approximately 100 to 150 numbers of larvae of a single genus were available as a sum from two to three sites during a single collection, which made it possible for the entire larvicidal assay. During warm climate condition the numbers of larvae available were considerably high, and during the cold and rainy climate the number reduced enormously. The sampling was done at three days intervals thus helping to maintain the availability of larvae consistantly. The larvae collected were separated in to different genera based on the macroscopic and microscopic morphological studies, based on the "Identification of the U.S mosquito larvae - manual".

Collection of plants: The Fenugreek plant, since it is one of the leafy vegetables used in food by the localities, it is easily available in market and shops, and is purchased from the local market. The Nerium plant is commonly present in many places of Vellore city especially in houses, roadsides and offices as ornamental plant. Leaves were collected from the herbal garden at VIT University.

Preparation of aqueous extract: The fresh leaves were washed with distilled water and were allowed for shade dried for a week, which was further finely grinded in a mortar and pestle. Aqueous extract was extracted sox let apparatus with distilled water with equal percentage (W/V), i.e., 100 g of the fresh leaf is crushed with 100 ml of distilled water; the extract collected were then allowed to evaporate in rotary vacuum evaporator. The collected concentrated aqueous phase was lyophilized and it is further diluted to the desired concentrations to perform the larvicidal assay.

Larvicidal assay: The Larvicidal assay was performed as described by the WHO. The larvae are released in different Petri plates of gradient concentration of the extract, and are observed after 24, 48 and 72 h, the larvae are tested for survival by touching with a needle and disturbing the Petri plate and if the larva does not moves after disturbing and touching, it is counted as dead (Abdul *et al*., 2008; Bishnu and Zeev, 2005; Das *et al*., 2007; Badruddoza, 2008; Nandita *at al*., 2008). The assay is performed thrice to get the mean value and standard deviation, by keeping the aqueous extract concentration in different percentage, diluted from the stock.

In addition to the Larvicidal activity of the plants Fenugreek and Nerium extracts individually, both the extracts are mixed in an equal proportion (1:1), and mixed well for both the extracts to interact well, and the larvicidal assay was performed.

The stock solution of the aqueous extracts are mixed in 1:1 ration to give 100% concentration stock, which is diluted to required concentrations. The stock extracts are mixed in 50ml: 50ml giving 100ml of 100% stock of combination extract. This is then diluted to required concentrations.

RESULTS

Mosquito survey in Vellore: The mosquito survey in Vellore city showed that, four genera of mosquitoes has been found, namely - Anopheles; Aedes; *Culex* and *Culiseta*. Which are identified based on their morphology, by "Identification of the U.S mosquito larvae - manual" (Atiya *et al*., 1995). Several other methods of classification has been considered, like e-classification, (John-e-Reinert, 2000), but, due to the simplicity, the former method was used.

In these three Aedes is predominantly found all over Vellore. Next to this, the *Culex* is equalizing the distribution with the Aedes, all over the city. The Anopheles is sparsely distributed in some regions and the *Culiseta* distribution is very scarce, being restricted to only some rural areas in the city, where the sanitation is worst.

The City regions have been divided into four sections for the study of the distribution of the mosquitoes and the distribution of the different genera are plotted in a statistically drawn overview map (Fig. 3). The first section lying in the North-Western part contains the *Culex* and Anopheles. The second section, lying towards the west consists of anopheles and Aedes. The Third section lying away from the centre (likely, towards the east), has all the four generas. The Fourth section, lying in the south east part also has all the four generas as well.

The distribution of all the four generas in the eastern part of this city may be due to presence of rural areas, with improper sanitation. With exception of the *Culiseta*, the other three types of the mosquitoes are present in large amount. While the other three types o f the mosquito larvae are available in large amount (>150/per collection), the *Culiseta* is very scarce (<20/per collection). The population of the *Culex* and *Aedes* are very high, and they are distributed in almost all part of the city.

Larvicidal assay of Aqueous extracts: As given in the Table 1, 2 and 3, both the plant's aqueous extracts has larvicidal activity for all three types of mosquitoes found in the survey. From the obtained data, it is visible that, being a toxic plant, the Nerium oleander has a less larvicidal activity than the nutritional plant, fenugreek

Table 1: The mean mortality of the larva with standard deviations for varying concentrations and exposure time for the *Anopheles* mosquito

Extracts	Concentration	Period of exposure		
		24 h	48 h	72 h
	1%	0±0	3±1	19±2
Nerium	2%	0±0	6±1	31±1
	3%	0±0	11±2	50±3
	1%	0±0	0±0	5±1
Fenugreek	2%	0±0	4±1	11±3
	3%	0±0	8±3	20±2
Combination	1%	12±2	20±3	38±1
	2%	37±1	57±2	71±2
	3%	53±3	100±0	100±0

Vellore District

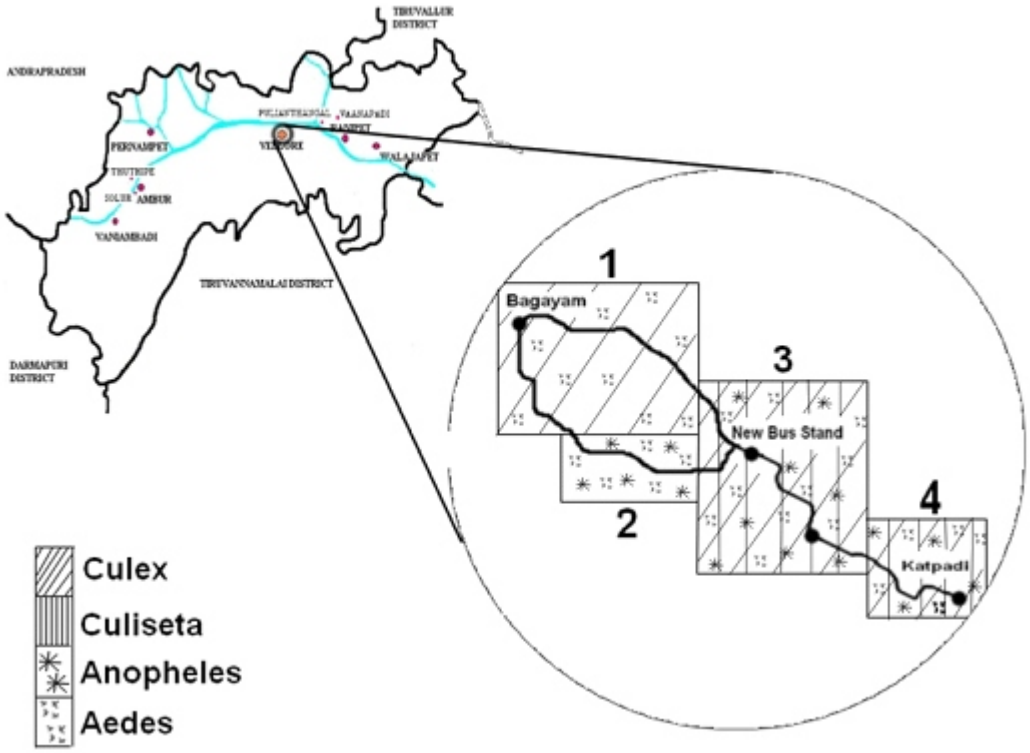

Fig. 3: The distribution of the four genera of mosquitoes in Vellore City

Table 2: The mean mortality of the larva with standard deviations for varying concentrations and exposure time for the *Aedes* mosquito

Extracts	Concentration	Period of exposure		
		24 h	48 h	72 h
	1%	54±3	100±0	100±0
Nerium	2%	79±2	100±0	100±0
	3%	91±1	100±0	100±0
	1%	81±2	100±0	100±0
Fenugreek	2%	100±0	100±0	100±0
	3%	100±0	100±0	100±0
	1%	78±2	100±0	100±0
Combination	2%	100±0	100±0	100±0
	3%	100±0	100±0	100±0

Table 3: The mean mortality of the larva with standard deviations for varying concentrations and exposure time for the *Culex* mosquito

Extracts	Concentration	Period of exposure		
		24 h	48 h	72 h
	1%	0±0	0±0	0±0
Nerium	2%	5±2	12±0	21±2
	3%	43±2	68±0	84±1
	1%	0±0	3±2	9±1
Fenugreek	2%	8±2	19±3	24±1
	3%	14±2	26±1	37±2
	1%	51±3	94±2	100±0
Combination	2%	78±1	100±0	100±0
	3%	100±0	100±0	100±0

(Trigonella foenum). Thus, the fenugreek plant is more effective for the larvicidal activity.

From the data obtained, it is also clear that, *Aedes* mosquito is more sensitive to both the plant extracts than the other two types. This proves that this type is more susceptible to the extracts.

Larvicidal assay of combined extracts: From the obtained data, it is clear that, the combined activity of both the extracts is more effective than the individual activity. In all the three types, the mortality rate increased considerably high when the extracts were combined together in an equal proportion.

The Fig. 4, 5 and 6, shows that almost all the three types of mosquitoes used in the study, are susceptible in an acceptable concentration, i.e., 3% concentration of the combined extract is capable of killing almost 100% in 3 days (72 h). The mortality rate was steadily increasing along with the time of exposure and concentration

DISCUSSION

From the results we obtained, the combination effects of the extracts were much effective than the individual extracts. So use of this combination in mosquito control can be of greater use. Due to the less availability of Culiseta mosquito, the larvicidal assay for this genus was not possible. The huge number of larvaes of the three genera shows the enormous amount of mosquitoes present

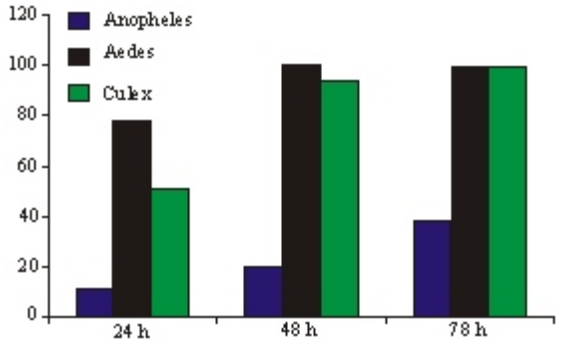

Fig 4: Comparison of the mean mortality of larvaes for 1% of the extract combination at 24, 48 and 72 h

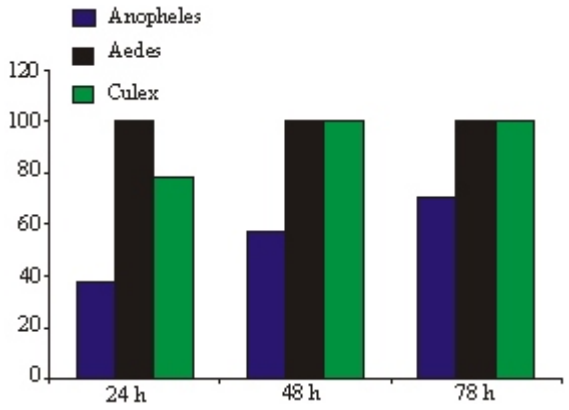

Fig 5: Comparison of the mean mortality of larvaes for 2% of the extract combination at 24, 48 and 72 h

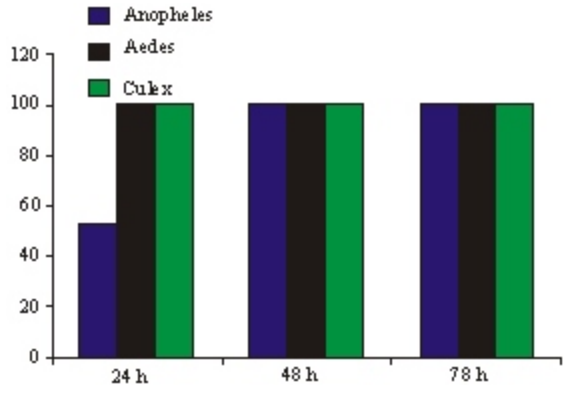

Fig 6: Comparison of the mean mortality of larvaes for 3% of the extract combination at 24, 48 and 72 h

in this locality. Thus, an immediate and effective step has to be taken to protect the public and environment.

Among the three tested larvae, the Aedes was the most sensitive one and showed the highest mortality, next is the Culex, which showed moderate mortality and the Anopheles with the low mortality than the two former genera. Though the Culiseta was not available in sufficient number to complete the assay, primary study about their mortality with the same procedure was performed. It showed that, Culiseta showed nil mortality for the extracts. 20 numbers of larvae were tested per concentration (1, 2 and 3%) and after 72 h the larvaes remained alive, proving to have the greatest tolerance among the other three larvae studied.

CONCLUSION

The primary objective of this work is to find a biological way of solution for the problem caused by the mosquito to the public. This method of aqueous extract preparation can be performed in houses and can be performed by any individuals, with a little care in handling the Nerium, so as to control the mosquito in the surrounding area. If this method is applied in use, considerable decrease in the mosquito can be brought in a short period of time.

With the above results obtained, we propose that these medically valuable plants, contains active compounds that are able to kill the mosquito larvae effectively, there by controlling the multiplication of the mosquitoes and also to eliminate the mosquitoes from a particular zone. This can be further used in the field test and we hope that there will not be any eutropication of bacteria or fungi due to this extracts because, these plants are already proved to contain antibacterial and antifungal activities in many research articles. So these compounds can be used as a solution for the long lasting mosquito problem in the developing countries without damaging the environment, and if these are marketed for the public reach, the use of the insecticidal sprays and liquidators for the control of mosquitoes will be reduced and thereby we can prevent the environmental contamination and to an extent, prevent the ozone layer depletion. This entire work is based on two concepts, which are; (1) It is easier to kill the larvae present in the stagnant water than to kill the adult flyers, thus if one stage of the mosquito life cycle is interrupted, the survival of the mosquitoes will be hard and can surely lead to complete elimination of the mosquitoes in a zone or area; (2) The plant extract (biological substances) are easily degraded and will not contaminate the environment, thus has greater advantage over the chemical methods.

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