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A study on the insecticidal activity of *Trigonella foenum-graecum* L. seed extracts against the filarial vector Culex quinquefasciatus Say, 1823 (Diptera: Culicidae)

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Abstract
A preliminary study was conducted to observe the larvicidal property of *Trigonella foenum-graecum*(methi) seed extract in 5 different solvents namely: ethanol, methanol, acetone, chloroform and tap water from 3rd December 2023- 15th January,2024 in the Vidyasagar College laboratory against *Culex quinquefasciatus* mosquitoes. Out of these 5 solvents Trigonella extract in acetone and methanol showed highest larvicidal property at 400 ppm with percentage mortality of *Culex quinquefasciatus* larvae at 100%. According to Lc_{50} values, the acetone extract had highest mosquitocidal activity with dosage of 46.774 mg/L. According to Lc_{90} values then also acetone extracthad highest mosquitocidal activity with dosage of 144.54 mg/L.

Keywords: Insecticidal activity, methi seed extract in organic solvents, Lc_{50} and Lc_{90}, *Culex quinquefasciatus*

Introduction
Mosquitoes of different species are vectors of diseases such as dengue, malaria, congenital Zika syndrome, lymphatic filariasis, yellow fever, West Nile fever, chikungunya, among many others. *Plasmodium* alone causes 212 million human malaria cases annually, mainly in tropical countries [3]. Malaria has killed more people than any other disease in the course of time.Until this century, the battle against the parasite was badly one-sided. Then, between 2000 and 2015, malaria cases dropped by a third worldwide, and mortality decreased by nearly half, because of widespread use of insecticide inside homes, insecticide-coated bed nets and better treatments. In addition to the damage caused by diseases transmitted by mosquitoes, these insects are responsible for a series of non-lethal impacts to the daily lives of individuals living in mosquito-infested areas due to the bites that cause pain, allergic reactions, besides other physical and psychological discomforts, which disrupt people's work activities and sleep quality [2].
There are many ways to control mosquito larvae but mostly they are not fully effective. There are mechanical, chemical, biological methods.
Fogging is one of the mechanical control methods. The target of the fogging is to kill adult mosquitoes. Unfortunately, fogging is considered less effective because it tends to repel mosquitoes from the nest, not kill mosquitoes. Chemical method is most widely used method to control mosquito larvae but it is not always effective since the vectors are transitory as they develop resistance against the chemical insecticides. Also, there are many environmental issues that arises because of using chemical pesticides. Hence, there has been an increasing interest in the development of alternative methods less hazardous to humans and other organisms. In this regard, plant-derived compounds have emerged as good candidates, not only as effective new tools in vector management but also as environmentally safer agents [4].
Proteinases are involved in the activation of proenzymes, blood coagulation, the digestion of fibrin clots, the processing and membrane transport of secretory proteins, germination, senescence, the defence against plant pathogens (especially fungi and insects), and the acquisition of nutrients and apoptosis [5]. Derivatives from many plant species have exhibited mosquitocidal activities [6, 7, 8].
As an alternative to chemicals, many scientists are trying to use biodegradable products to use as larvicidal agent. So, the present study was designed to access the larvicidal property of *Trigonella foenum-graecum* (Fabaceae) seed extract in 5 organic solvents such as ethanol, methanol, acetone, chloroform and tap water against the mosquito larvae of *C. quinquefasciatus.*

Materials and Methods
1. Experimental location
This experiment was conducted in the entomology laboratory of Vidyasagar College, Salt Lake Campus, Kolkata under ambient laboratory conditions of 30 ± 2°C temperature, 75 + 5% relative humidity.

2. Collection of test mosquitoes larvae
The present study was conducted to prove the larvicidal potency of methi seed extracts in 5 organic solvents against *Culex quinquefasciatus* Say, 1823 larvae. *Culex* spp. mosquito larvae were collected from drains surrounding the Vidyasagar College campus and were brought into the laboratory. The *C. quinquefasciatus* mosquito larvae were identified by their morphological characters. Larvae of *C. quinquefasciatus* mosquito were kept in separate plastic containers and they were fed artificial food i.e., mixture of dog biscuits and dried yeast powder in the ratio 3:1. The culture was maintained and reared in laboratory. Also, the colonies were kept free from exposure to pathogen, insecticides and repellents.

3. Preparation of seed extract
Seeds from plants *Trigonella foenum-graecum* (methi) were collected and sun dried for 3 weeks. After sun drying the seeds for 3 weeks the seeds were cracked to remove their endocarps and was again sun dried for 1 week. Then the dried seeds were separately powdered using a stainless blender and each kept separately in different petri dishes. Now they are extracted by using 5 different solvents: ethanol, methanol, acetone, chloroform and tap water. Each

seed extract was put in 5 different solvents in conical flasks. These solutions were kept for 3 days in room temperature in the laboratory. The conical flasks were stirred gently for 3 times each day. After 3 days each solution was poured in different petri dishes and were put inside an incubator at a temperature of 40° C in order to remove the extracting solvent.

4. Larvicidal bioassay

Then the dried seed extracts were kept in separate containers and refrigerated for 2 days. After 2 days the dried seed extracts were taken and measured 0.10g on the weight balance. The larvicidal bioassay followed the World Health Organization (WHO) standard protocol with a slight modification. Aqueous extract of the sample at 5 concentrations (25,50,100,200,400ppm) were applied for bioassay experiment. The already prepared concentrations were transferred to small petri dishes each marked with that particular concentration. 10 *Culex quinquefasciatus* mosquito larvae are given to each concentration of each seed extract in each solution. Control was performed using only tap water and no seed extract. No food was provided to the larvae. Larvae were considered dead if they were unrousable within a period of time even if they were gently prodded. Larva mortality was observed after 24hrs, 48hrs,72hrs and 96hrs.

5. Statistical analysis

Statistical analysis of the experimental data was performed by using MS Excel 2020 and anti-log calculator to calculate LC50 lethal dose, LC90 lethal dose, regression analysis, co-efficient values, mean larval mortality, standard error and so on.

Results

The *Trigonella foenum-graecum* seed extracts in 5 solvents showed significant larvicidal activity against *Culex quinquefasciatus*. The Table 1 shows that the methanol extract at 400 ppm and acetone extract at 400 ppm showed maximum mortality of 100%. Thus, Trigonella sp. In methanol and acetone extract had highest larvicidal property. In table 1, after 24 hrs,48 hrs and 72 hrs only the acetone extract showed highest larvicidal activity among the other solvents. The tap water extract showed least larvicidal properties at each concentration. Ethanol and methanol extracts showed moderate larvicidal property.

Table 1: Percentage Mortality of *Culex quinquefasciatus* larvae in meth seed extract in 5 organic solvents

Solvent	Concentration (ppm)	No. of larvae	Mortality Rate			
			24hrs	48hrs	72hrs	96hrs
1. ethanol	25	10	0%	0%	6.67%	16.67%
	50	10	0%	6.67%	13.33%	23.3%
	100	10	6.67%	23.33%	36.67%	46.6%
	200	10	10%	30%	43.33%	60%
	400	10	16.67%	36.67%	60%	73.33%
	Control	10	0%	0%	0%	0%
2. Methanol	25	10	3.33%	13.33%	20%	26.67%
	50	10	10%	23.33%	36.67%	53.33%
	100	10	13.33%	40%	53.33%	66.67%
	200	10	16.67%	43.33%	60%	76.67%
	400	10	26.67%	53.33%	80%	100%
	Control	10	0%	0%	0%	0%
3. Acetone	25	10	0%	6.67%	20%	33.33%
	50	10	3.33%	30%	43.33%	56.66%
	100	10	10%	36.66%	60%	66.67%
	200	10	23.33%	46.67%	73.33%	83.33%
	400	10	33.33%	66.67%	86.67%	100%
	Control	10	0%	0%	0%	0%
4. Chloroform	25	10	0%	3.33%	10%	26.67%
	50	10	6.67%	20%	36.67%	46.67%
	100	10	10%	33.33%	46.67%	66.67%
	200	10	26.67%	40%	56.67%	73.33%
	400	10	30%	60%	76.67%	83.33%
	Control	10	0%	0%	0%	0%
5. Tap water	25	10	0%	0%	3.33%	10%
	50	10	0%	3.3%	16.67%	23.33%
	100	10	3.33%	20%	33.33%	40%
	200	10	3.33%	23.33%	36.67%	50%
	400	10	10%	30%	43.33%	63.33%
	Control	10	0%	0%	0%	0%

The efficiency of *Trigonella foenum-graecum* extract in 5 different solvents, is calculated by using the standard error calculator as seen in Table 2.

It was observed during this study that as the time proceeds from 24hrs to gradually 96 hrs the Lc_{50} and Lc_{90} value of each extract gradually decreases (Table3). The acetone extract has the lowest Lc_{50} value and hence it has the maximum larvicidal

property. According to Lc_{90} value also the acetone extract has highest larvicidal property followed by methanol extract, chloroform extract, ethanol extract and finally tap water extract (Table3).

Table 2: Efficiency of *Trigonella* sp. seed extract in 5 organic solvents against *Culex quinquefasciatus* mosquito larvae

Solvent	Conc. (ppm)	Mortality Rate ±SE			
		24hrs	48hrs	72hrs	96hrs
1. Ethanol	25	0 ± 0	0 ± 0	0.67± 0.33	1.67± 0.33
	50	0 ± 0	0.67± 0.33	1.33± 0.33	2.33 ± 0.66
	100	0.67± 0.33	2.33 ± 0.33	3.67± 0.33	4.67± 0.33
	200	1 ± 0	3 ± 0	4.33 ± 0.33	6± 0
	400	1.67 ± 0.33	3.67 ± 0.33	6 ± 0.57	7.33 ± 0.33
2. Methanol	25	0.33 ± 0.33	1.33 ± 0.33	2 ± 0	2.67 ± 0.33
	50	1± 0.57	2.33± 0.33	3.67± 0.57	5.33± 0.33
	100	1.33± 0.33	4± 0	5.33± 0.66	6.67± 0.33
	200	1.67± 0.88	4.33± 0.33	6± 0	7.67± 0.33
	400	2.67 ± 0.33	5.33 ± 0.33	8 ± 0.57	10 ± 0
3. Acetone	25	0 ± 0	0.67 ± 0	2 ± 0.57	3.33 ± 0.33
	50	0.33± 0.33	3± 0	4.33 ± 0.33	5.67± 0.33
	100	1± 0.57	3.67± 0.33	6± 0	6.67± 0.33
	200	2.33± 0.33	4.67± 0.33	7.33± 0.66	8.33± 033
	400	3.33 ± 0.66	6.67 ± 0.33	8.67 ± 0.33	10 ± 0
4. Chloroform	25	0 ± 0	0.33 ± 0.33	1 ± 0	2.67 ± 0.33
	50	0.67± 0.33	2± 0.57	3.67± 0.33	4.67± 0.33
	100	1± 0.57	3.33± 0.33	4.67± 0.33	6.67± 0.33
	200	2.67± 0.33	4± 0	5.67± 0.33	7.33± 0.33
	400	3 ± 0	6 ± 0.57	7.67 ± 0.33	8.33 ± 0.33
5. Tap Water	25	0 ± 0	0 ± 0	0.33 ± 0.33	1 ± 0
	50	0 ± 0	0.33 ± 0.33	1.67 ± 0.33	2.33± 0.33
	100	0.33 ± 0.33	2 ± 0	3.33± 0.33	4± 0
	200	0.33± 0.33	2.33± 0.33	3.67± 0.33	5± 0.57
	400	1 ± 0	3 ± 0.57	4.33 ± 0.33	6.33 ± 0.33

Table 3: Log probit analysis and regression analysis of larvicidal activity of methi seed extract in 5 organic solvents on *Culex quinquefasciatus* mosquito larvae

Solvent	Time	Regression Equation	r^2	LC_{50}	LC_{90}	P value	Upper limit	Lower limit	Standard error
Ethanol	24 hrs	Y= 1.2390x + 0.825	0.929	2290.9	25119	0.008	1.865	0.612	0.187
	48 hrs	Y= 1.6510x + 0.598	0.905	457.09	2754.20	0.012	2.633	0.669	0.293
	72 hrs	Y= 1.5114x + 1.3830	0.967	245.47	1737.8	0.002	2.020	1.002	0.152
	96 hrs	Y= 1.3918x + 2.022	0.984	134.90	1122	0.0008	1.717	1.066	0.097
Methanol	24 hrs	Y= 0.953x + 1.923	0.936	1659.6	37154	0.006	1.410	0.496	0.136
	48 hrs	Y= 0.989x + 2.576	0.944	281.84	5495.4	0.005	1.430	0.549	0.131
	72 hrs	Y= 1.308x + 2.382	0.976	100.23	954.99	0.0014	1.678	0.939	0.111
	96 hrs	Y= 2.677x+ 0.393	0.825	52.481	154.88	0.032	4.940	0.414	0.676
Acetone	24 hrs	Y= 1.634x + 0.397	0.989	645.65	3981.1	0.0004	1.936	1.332	0.902
	48 hrs	Y= 1.421x + 1.762	0.918	186.21	1479.1	0.0100	2.197	0.645	0.232
	72 hrs	Y= 1.571x + 2.051	0.989	75.336	489.78	0.0046	1.870	1.272	0.089
	96 hrs	Y= 2.601x + 0.642	0.838	46.774	144.54	0.028	4.695	0.506	0.626
Chloroform	24 hrs	Y= 1.491x + 0.772	0.932	676.08	4897.8	0.007	2.230	0.752	0.220
	48 hrs	Y= 1.611x + 1.145	0.918	245.47	1513.6	0.010	2.494	0.728	0.264
	72 hrs	Y= 1.511x + 1.823	0.934	125.89	870.6	0.007	2.246	0.776	0.219
	96 hrs	Y= 1.265x + 2.730	0.965	61.660	630.96	0.002	1.705	0.826	0.131
Tap water	24 hrs	Y= 0.847x + 1.365	0.870	19498	6.3096e+5	0.020	1.447	0.246	0.179
	48 hrs	Y= 1.581x + 0.575	0.897	616.6	3981.1	0.014	2.561	0.601	0.293
	72 hrs	Y= 1.335x + 1.573	0.844	316.23	3311.3	0.027	2.389	0.281	0.315
	96 hrs	Y= 1.315x + 1.981	0.977	194.9	1584.9	0.001	1.684	0.946	0.110

Discussion

Mosquito control at the larval stage is effective since they are restricted within their own habitat, found in an aquatic environment unlike flying adults. Also, the present study also shows that the seed extracts have very less harmful effect on the non-target organisms. Since the last four decades, many scientists have used seed and leaf extracts for their larvicidal properties. Some scientists have also used seed oil and it showed high larvicidal properties [9].

In this study, we can see the methanol extract at 400 ppm and acetone extract at 400 ppm showed maximum mortality of 100%. The acetone extract has the lowest Lc_{50} value of 46.744 mg/L and hence it has the maximum larvicidal property. According to Lc_{90} value also the acetone extract (144.54mg/L) has highest larvicidal property [Table1-3].

A similar study was conducted by Thongwat *et al.* [9] to find the larvicidal activity of endocarp and seed crude extracts of *Dracaena loureiri* against Aedes aegypti mosquito. The

larvicidal bioassay in this study demonstrated that the ethanolic endocarp extract was the most effective with the LC50 value of 84.00 mg/L after 24 h exposure and < 50 mg/L after 48h exposure.

Conclusion

Mosquito control is very important as mosquitoes are solely responsible for diseases like malaria, yellow fever, dengue-haemorrhagic fever and so on.With the loss of effective insecticides for many vector species, integrated approaches have been promoted to improve control, manage resistance, reduce unintentional impact of insecticides and engage communities in healthy behaviours [1]**.** Scientists are trying to find alternatives for control of mosquito larvae which are less harmful for environment, humans as well as non-target organisms. From this present study it is concluded that the methi seed extracts in ethanol, methanol, acetone, chloroform and tap water can be recommended for beneficial mosquito larvae control.

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