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Toxicity of *Semecarpus anacardium* L. seed extracts against immature stages of *Culex quinquefasciatus* Say and *Aedes aegypti* L. (Diptera: Culicidae)

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Abstract
The present study was conducted to evaluate the larvicidal and ovicidal activities of various extracts of *Semecarpus anacardium* seeds against *Culex quinquefasciatus* Say and *Aedes aegypti* L. mosquitoes. This experiment was carried out at Entomology Research Institute, during the year 2015-2016. Extracts were prepared different concentrations at 62.5, 125, 250 and 500 ppm using acetone for larvicidal and ovicidal activities. The lethal concentration (LC) values were calculated by probit analysis software (SPSS 11.5 version). The ovicidal activity results were analyzed using the Graph Pad Prism 5.0. After 24 h of treatment the hexane extract showed the highest larvicidal activity with LC_{50} and LC_{90} values of 36.75, 234.057 ppm and 48.88, 299.56 ppm concentrations against the larvae of *Cx. quinquefasciaus* and *Ae. aegypti*, respectively. Similarly the same hexane extract showed 94.97% and 83.92% ovicidal activity at 500 ppm concentration after 120 h post treatment. The result clearly indicates that hexane extract of *S. anacardium* seed could be used to control these vector mosquitoes.

Keywords: *Semicarpus anacardium*, larvicidal activity, ovicidal activity, *Culex quinquefasciatus*, *Aedes aegypti*, lethal concentrations

Introduction
Mosquitoes transmit various diseases to humans; hence, they are called 'public enemy number one' [1]. Mosquito borne diseases are considered as the most dangerous human diseases responsible for very high number of deaths every year. In tropical and subtropical countries, *Culex quinquefasciatus* is a major vector involved in the transmission of nematode worm *Wuchereria bancrofti* responsible for lymphatic filariasis [2]. According to the World Health Organization (WHO) report, there are 128 million people over 78 countries around the world affected by lymphatic filariasis [3, 2]. The mosquito *Ae. aegypti* L. is the major vector of chikungunya and dengue fever; it is endemic in the Asian countries including India [4]. In India hundreds of deaths were caused by dengue fever in 2012 and the number of dengue patients is increasing year by year [5-7].
These two major vectors, namely *Cx. quinquefasciatus* and *Ae. aegypti*, need to be controlled. Many synthetic insecticides, viz., pyrethroids, organophosphates, organochlorines and carbamates are used in mosquito control programme. Environmental problems, development of pesticide resistance, destruction of non-target organisms and human health problems were reported for these chemical insecticides [8-14, 7, 15]. Hence, alternative mosquito control methods are needed to protect environment and health [16-19]. Plant extracts are one of the natural sources in mosquito control.
Semecarpus anacardium L. (Family: Anacardiaceae) is recommended in various medical treatments for the cure of different diseases. It is distributed in tropical, sub-Himalayan region and central parts of India. It is used in indigenous medicines [20, 21, 15]. Earlier, phytochemical analyses of *S. anacardium* nut revealed the presence of biflavonoids, phenolic compounds, bhilawanols, minerals, vitamins and amino acids [22]. Further, *S. anacardium* is classified in Ayurveda under the category of toxic plants [23]. Nuts of *S. anacardium* are used in the Ayurveda and Siddha systems of medicine, with various therapeutic properties, such as anti-helminthes, antifungal, anticarcinogenic, nervous debilities, arthritis and cardiovascular disease [24].

There are no reports available on the bioactivities of seed extracts of *S. anacardium* against vector mosquitoes. Hence, the present study was undertaken to screen the seed extracts of *S. anacardium* for their larvicidal and ovicidal activities against *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes.

Materials and Methods

Plant material

This study was conducted at Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India during the year 2015-2016. The dry seeds (500 g) of *S. anacardium* are purchased from seed suppliers in Parrys, Chennai. The seeds were identified by a taxonomist and the voucher specimen (*S. anacardium:* ERI-BP-GS-003) was deposited at the herbarium of the Entomology Research Institute.

Extraction

Dried seeds of *S. anacardium* were coarsely powdered using an electric blender. Sequential extraction of seed powder (500 g) was done by successive soaking of the seed powder in 1.5 L of hexane, chloroform, ethyl acetate and methanol for a period of 48 h. The extracts were filtered through Whatman No 1 filter paper and concentrated in a rotary evaporator and finally dried in vacuum and stored at 4 °C.

Larvicidal assay

Larvicidal activity was evaluated using a modified method prescribed by the [25]. Twenty early fourth-instar larvae of *Cx. quenquefasciatus* and *Ae. aegypti* were introduced into the containers. The different concentrations viz., 62.5, 125, 250 and 500 ppm for each extract were prepared using acetone. Acetone control and water control were also maintained separately. Five replicates were maintained for each concentration and control. Larvae were considered dead when they did not respond to stimulus or did not rise to the surface of the treatment solution. Mortality was registered after 24 h exposure period. The percent mortality was calculated using the formula (A.1), and corrections for mortality when necessary were done using Abbott's [26] formula (A.2).

A.1

$$\text{Percentage of Mortality} = \frac{\text{No. of Dead larvae}}{\text{No. of Larvae introduced}} \times 100\%$$

A.2

$$\text{Abbott's corrected mortality} = \frac{\text{\% mortality in treatment - \% of mortality in control}}{\text{100 - \% of mortality in control}} \times 100\%$$

Ovicidal assay

The ovicidal activity was assessed following the method of Elango *et al.* [27]. Test concentrations 62.5, 125, 250 and 500 ppm were prepared using acetone. Twenty five freshly laid eggs of *Cx. quinquefasciatus* and *Ae. aegypti* were exposed to each concentration prepared acetone. Each concentration was replicated five times. Acetone control and water control were maintained separately. The ovicidal rate was assessed in percentage at 120 h post treatment using the following formula (A.3).

A.3

$$\text{Ovicidal activity (\%)} = \frac{\text{Number of unhatched eggs}}{\text{Total number of eggs exposed}} \times 100\%$$

Statistical analysis

The lethal concentration values (LC_{50} and LC_{90}) were calculated by probit analysis software (SPSS 11.5 version). The calculated ovicidal activity results were analyzed using the Graph Pad Prism 5.0 software package.

Results

The results of larvicidal activity clearly showed that hexane extract of *S. anacardium* seed was the most effective treatment against *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes. The LC_{50} values of hexane extract of *S. anacardium* were 36.75 ppm, 48.88 ppm and the LC_{90} values were 234.057 ppm, 299.56 ppm against *Cx. quinquefasciatus* and *Ae. aegypti* larvae, respectively (Table 1). Similarly the same hexane extract showed significant ovicidal activity of 94.9 and 83.9% at 500 ppm concentration against *Cx. quinquefasciatus* and *Ae. aegypti* eggs, respectively (Fig.1 and 2). The chloroform extract showed moderate larvicidal and ovicidal activities against both mosquito species. The ethyl acetate and methanol extracts showed very low activity even at 500 ppm concentration against both vector mosquitos.

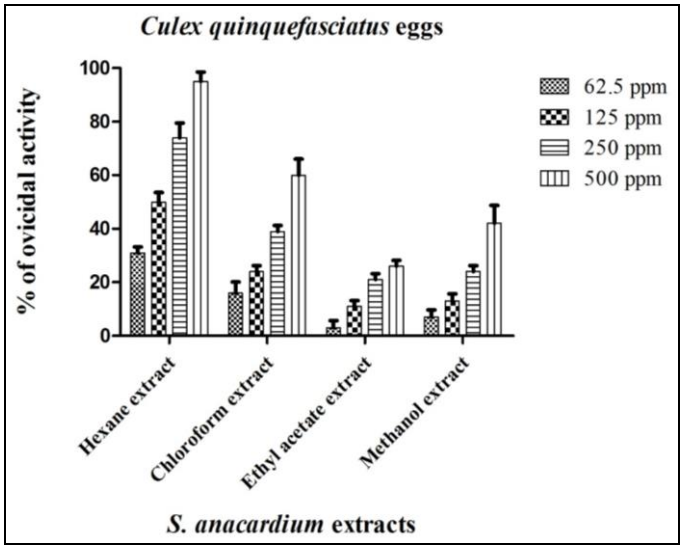

Fig 1: Percent ovicidal activity of *S. anacardium* seed extracts against *Cx. quinquefasciatus* eggs

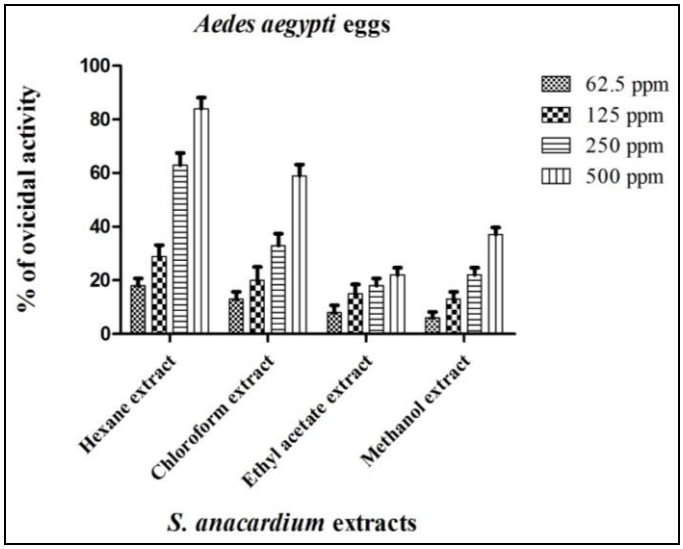

Fig 2: Percent ovicidal activity of *S. anacardium* seed extracts against *Ae. aegypti* eggs

Table 1: Lethal concentrations (in ppm) of *S. anacardium* seed extracts against *Cx. quinquefasiatus* and *Ae. aegypti* larvae.

Mosquito species	Solvent extracts	LC_{50} (ppm)	95% confidential limit		LC_{90} (ppm)	95% confidential limit		Intercept ± SE	X^2
			Lower limit (ppm)	Upper limit (ppm)		Lower limit (ppm)	Upper limit (ppm)		
Cx. quinquefasiatus	Hexane	36.75	0.94	61.39	234.057	207.415	273.60	-.24±0.06	45.65
	Chloroform	197.20	133.76	253.01	758.92	616.03	1039.66	-0.45±0.05	77.72
	Ethyl acetate	466.16	382.50	382.50	1180.52	914.34	1775.50	-.84±0.05	65.12
	Methanol	560.85	486.81	678.86	1158.86	968.52	1481.21	-1.20±0.06	38.84
Ae. aegypti	Hexane	48.88	-18.35	90.08	299.56	250.38	386.97	-0.25±0.05	118.70
	Chloroform	312.17	274.92	356.76	772.47	670.86	928.32	-0.86±0.05	48.65
	Ethyl acetate	703.36	577.18	980.46	1150.72	903.55	1722.54	-2.01±0.08	102.22
	Methanol	669.96	-	-	809.93	-	-	-6.13±2.88	21.361

Discussion

Synthetic chemical pesticides are responsible for the development of pesticide resistance in mosquitoes and in many other insect pests. In addition, they are toxic to human beings and other beneficial organisms. These showed the need for ecofriendly and plant based pesticides for mosquito control [28, 7]. Plant derived extracts/insecticides may be a good alternative to synthetic insecticides as they are nonhazardous to the environment and other organisms. Literature reveals that much research on plant-based pesticides is going on and that plant extracts are good alternatives to fight against vector mosquitoes [29, 30, 9]. Some plant-based biocides such as Arborine, Capillin, Goniothalamin, Neolignans, Ocimenone, Quassin, Rotenone, etc. have been chemically synthesized and are widely used in mosquito control programmes [31, 9].

In the present study, the toxicity of *S. anacardium* seed extracts was screened against *Cx. quinquefasciatus* and *Ae. aegypti*. The highest mortality was recorded in the hexane extract treatment against both mosquito vectors. Reegan *et al.* [6] have reported that hexane extract of *Limonia acidissima* leaves was toxic against the larvae of *Cx. quinquefasciatus* and *Ae. Aegypti*. Earlier we have reported the phytochemical analysis of hexane extract of the *S. anacardium* seeds [15], which showed the presence of steroids and terpenoids. These phytochemical groups might be responsible for the larvicidal activity in the present study. Rawani *et al.* [9] have reported that aqueous extract of *Carica papaya* seeds was toxic against *Cx. quinquefasciatus* larvae and stated that many bioactive principles such as alkaloids, steroids, terpenes and saponins in *C. papaya* seed extracts might be responsible for the larvicidal activity.

The LC_{50} values of hexane extract of *S. anacardium* were 36.75 and 48.88 ppm against *Cx. quinquefasciatus* and *Ae. Aegypti* respectively. This result is similar to the earlier study by Elango *et al.* [32] who have reported that the hexane extract of *Andrographis paniculata* recorded low LC_{50} values of 67.24 and 88.50 ppm against *An. subpictus* and *Cx. tritaeniorhynchus*, respectively.

Several investigators have reported the mosquito larvicidal activity of plant seeds. Sakthivadivel and Thilagavathy [33] have reported that the acetone fraction of petroleum ether extract of *A. mexicana* seeds had larval toxicity against second instar larvae of *A. aegypti* at 25, 50,100 and 200 ppm concentrations. Similarly [34] Schmutterer and [35] Senthil Nathan *et al*. have shown that neem and other plants seeds were toxic to larvae and affected fecundity and fertility of vector mosquitoes. Abdul Rahuman *et al*. [36] evaluated the hexane extracts of *Dirca palustris* seeds and found that it was toxic to fourth instar *A. aegypti* larvae. Sivaraman *et al.* [15] have reported the larvicidal activity of chloroform extract of *H. pentandra* seeds against *Ae. aegypti* and *Cx. quinquefasciatus*; the LC_{50} values were 89.52 and 248.28 ppm for *Cx. quinquefasciatus* and *Ae. aegypti* respectively. These concentrations were higher than *S. anacardium* in the present study.

In the present study, all the four solvent extract treatments were not equally effective against *Cx. quinquefasciatus* and *Ae. aegypti*. *Cx. quinquefasciatus* life stages were found to be more susceptible to the treatments than *Ae. aegypti*. Similar species specific toxicity of plant extracts against mosquito larvae was reported by Martınez *et al.* [37] who found that ethanol extract of marine sponge *Amphimedon compressa* was more toxic against the larvae of *Ae. aegypti* than *Cx. quinquefasciatus*.

Conclusion

In conclusion, the hexane extract of *S. anacardium* seed showed very good larvicidal and ovicidal activities against *Cx. quinquefasciatus* and *Ae. aegypti* vector mosquitos. Based on these results, the hexane extract of *S. anacardium* seed can be further probed to isolate the active principle responsible for the toxic effect.

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