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Studies on the Control of Mosquito, *Aedes Aegypti* using the Ethanol Seed Extract of *Annona Reticulata* As A Biocide

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ABSTRACT

In the present study the seeds of *Annona reticulata* was extracted with ethanol, and evaluated for mosquitocidal effect against the dengue vector *Aedes aegypti*. The preliminary phytochemical analysis showed the presence of alkaloids, flavanoids, glycosides, steroids and phenols in the ethanol seed extract of *Annona reticulata*. The percentage of egg hatchability in ethanol seed extract was 73% in 0.1%, 59% in 0.2% 42% in 0.3%, and 25% in 0.4%, 11% in 0.5% respectively and 100% hatchability was seen in control. The level of the toxicity of *Annona reticulata* was expressed in terms of LC50/24 hour value. The LC50 values of ethanol seed extract for I, III & IV instar larvae and pupae of *Aedes aegypti* were 0.717 %, 1.263 %, 1.369 %, 1.425 % respectively. The morphogenetic abnormalities are commonly caused by botanical extracts and the disturbance from the growth regulating hormones. It is therefore suggested that *Annona reticulata* derivatives are considered for vector control operations besides their use in other fields after exploring field trails.

Keywords : *Annona reticulata, Aedes aegypti*, Larvicidal effect, Insecticide.

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INTRODUCTION

Mosquitoes are the oldest human enemy and represent a significant threat to human health because of their ability to vector pathogens that cause disease that afflict million of people worldwide[1]. Several species belonging to genera *Aedes, Anopheles and Culex* are vector for the pathogens of various diseases like malaria, filariasis, yellow fever, dengue fever, dengue haemorrhagic fever and Japanese encepahalitis[2]. Mosquitoes borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally and 40,000,000 of the Indian population[3].Mosquito-borne disease have an economic impact including loss in commercial and labour out puts particularly in countries with tropical and sub tropical climates however, no part of the world is free from vector-born disease [4].

Aedes aegypti mosquitoes are originally found in tropical and sub tropical zones, but now found on all continents excluding Antarctica. *Aedes aegypti* is a very important disease vector transmitting the arbovirus causing dengue haemorrhagic fever (DHF) and chikungunya in human. At present, no effective vaccine is available for dengue therefore, the only way of reducing the incidence of this is by mosquito control which is frequently dependent on applications of conventional synthetic insecticides[5]. Controlling mosquitoes is of utmost importance in the present day scenario with rising number of mosquito borne disease. Extensive application of chemical insecticides for many years in this area caused irreparable damage to environment and resulted in resistance among mosquitoes. Improper dosage and faulty application of synthetic insecticides result resurgence of secondary pest population and increase resistance to disease vector. There is argent need to find alternatives to the synthetic insecticides which is more potent and low cost [6].

Plants are a rich source of bioactive organic chemicals and synthesize a number of secondary metabolites that serve as insecticides, anti-feedent, oviposition deterrents, adulticides, repellents, growth inhibitors[7].These plant act as rich source of natural compounds exhibiting medicinal insecticidal and many other properties and are well known to be most effective with least side effect. Plant products are given importance due to their biodegradable nature easy available and no effect on non-target organism that share the some environmental guild with mosquito larval[8]. The natural products, especially plant derivatives called bio-insecticides are now emerging as a visible component of Integrated Pest Management (IPM) strategies in view of their pesticidial potency as well as efficacy on pests and pests and safely to parasitoids and predators[9].The primary objective of this work is to find a biological way of solution using seed extract of *Annona recticulata* for the problem caused by the mosquito to the public.

MATERIALS AND METHODS

For the present study the ethanolic extract of *Annona reticulata* was used to test the efficacy and insecticide effects against different development stages of the mosquito *Aedes aegypti* (Diptera: culicidae).

REARING AND MAINTANANCE OF THE MOSQUITOES

Collection of eggs

The eggs of *Aedes aegypti* were collected from National Institute of Communicable Disease (NICD) at Mettupapalyam. The egg were placed separately in a sterilized glass through containing to liters of unchlorinated tap water, (Size 18cm diameter and 9cm height) in laboratory conditions.

Rearing of larvae, pupae and adult

After 24 hrs freshly hatched larvae were collected and maintained in separate containers with tap water (capacity 2liters) and glucose biscuit and yeast (2:1) was given as source of food. Pupae were isolated from the culture and were allowed to emerge into adults in the mosquito net cage (42 cm x 30cm x 30 cm). Emerged adults were fed with 10% sucrose solution socked in cotton wick[10]. Pigeon blood was given as a source of food for female mosquitoes as detailed by [11].

Different batches of adults were maintained in the cage by introducing sufficient number of pupae. A small container with water was kept inside the cage to facilitate the female to lay the eggs. The eggs in the container were removed carefully and allowed to hatch. Eggs are laid singly and placed on the dry surface water. Usually the eggs are laid early in the morning. They are oblong shaped. The incubation period of the normal eggs were 48 hours. The egg hatchability was examined every 6 hours (6, 12, 18 and 24 hours) and the number of larvae was recorded. Glucose biscuit was given as a source of food.

Preparation of phytochemical extract

The seeds collected from field were brought to the laboratory, then washed with water followed by distilled water and were dried under shade and the dried seeds were ground to fine powder. The dried seeds and powdered seeds (100 gm) were extracted with acetone (300 ml) by using Soxhlet apparatus for 8 hours[12].The extract was concentrated in a vacuum evaporator to yield dark greenish gummy extract. The residue was then made into 1% stock solution with acetone and taken for further bioassay test.

Preliminary phytochemical studies

The extract was subjected to determine the groups of secondary metabolites such as alkaloids, flavanoids, glycosides, saponins, tannins, steroids and phenols.

Bioassay test

To obtain different concentrations of test medium the crude extract, 1 to 10ml of the stock solution were dissolved in water and mixed thoroughly with the dry ingredients of the diet as suggested by Miller and Chamberlain. Freshly laid eggs, newly emerged, 1st, 3rd and 4th instar larvae and freshly moulted pupae of *Aedes aegypti* were exposed to different percent test concentrations of *Annona reticulata* and the results are presented in the table 1,2,3,4 and 5. Three replicates were done at a particular concentration. Controls were maintained by using ethanol test medium. Egg hatchability and mortality of different developmental stages (1st, 3rd and 4th instar larvae and pupae) of the treated and the control over a period of 24 hours was observed. Mortality in control was negligible. The mortality at the different concentrations, LC50 of the plant extract that can kill 50% of the treated stages of each treated was calculated and presented in the table 3.

RESULTS AND DISCUSSION

Today environmental safety of an insecticide is considered to be paramount importance. An insecticide does not have to cause high mortality on target organisms in order to acceptable[13].In the present study the dried seeds of *Annona reticulata* was extracted with ethanol and the extract was evaluated for mosquitocidal effect against *Aedes aegypti*. Identifying plant based insecticides that are efficient as well as suitable and adaptive to local ecological conditions, biodegradable and have the widespread insecticidal property will obviously work as a new weapon in the arsenal of insecticides and in the future may act as a suitable alternative product to fight against mosquito-borne diseases[14].

Table1 reveals the results of phytochemical screening of *Annona reticulata*. Ethanolic extract showed the presence of alkaloids, flavonoids, glycosides, steroids and Phenols. Phytochemicals may serve as suitable alternatives to synthetic insecticides since the insecticide are relatively safe, inexpensive and are readily available in many areas of the world.

Table 1: Results of Preliminary Phytochemical Screening of *Annona Reticulata* Seed using Ethanol Extract

Secondary Metabolites						
Alkaloids	Flavonoids	Glycosides	Steroids	Tannins	Saponins	Phenols
+	+	+	+	-	-	+

+ indicates the presence of compounds and - indicates the absence of compounds

Effect of *Annona reticulata* on egg hatchability of *Aedes aegypti*

The freshly laid eggs of *Aedes aegypti* were exposed to 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% of ethanol seed extract of *Annona reticulata*. The percentage of egg hatchability in ethanol seed

Table 2: Ovicidal effect of *Annona reticulata* seed extract against *Aedes aegypti.*

No of larvae exposed	Concentration (%)											
	Control		0.1		0.2		0.3		0.4		0.5	
	Hatch	Un hatch	Hatch	Unhatch	Hatch	Unhatch	Hatch	Unhatch	Hatch	Unhatch	Hatch	Unhatch
20	20	0	13	7	11	9	7	13	4	16	3	17
20	20	0	16	4	12	8	6	14	8	12	2	18
20	20	0	15	5	10	10	9	11	4	16	2	18
20	20	0	14	6	14	6	10	10	5	15	1	1
20	20	0	15	5	12	8	10	10	4	16	3	17
Mean	20	0	14.6	5.4	11.8	8.2	8.4	11.6	5	15	2.2	1.8
SD±	0	0.00	1.14	1.14	1.48	1.48	1.82	1.82	1.73	1.73	0.84	0.84
Mean(%)	100	0	73	27	59	41	42	58	25	75	11	89

Table 3: LC50 values of ethanolic seed extract of *Annona reticulata* on larvae and pupae of *Aedes aegypti*.

Larval and pupal stages	% of mortality						LC50 (%)	Regression equation	Chi-Square
	Control	0.5%	1.0%	1.5%	2.0%	2.5%			
I-Instar	0	22	29	57	100	100	0.717(0.059-1.303)	Y=1.306+1.820x	29.382
III-Instar	0	26	52	76	100	100	1.263(0.895-1.637)	Y=39.4x-0.3	0.384
IV-Instar	0	20	35	57	76	96	1.369(1.685-1.042)	Y=38.6x-1.1	0.209
Pupae	0	10	14	20	40	90	1.425(0.852-3.148)	Y= -1.795+1.259x	28.503

extract was 100% in control, 73% in 0.1% concentration, 59%in 0.2% concentration, 42% in 0.3% concentration and 25% in 0.4% and 11% in 0.5% concentration. These values are statistically significant at 5% level (Table 2). The egg hatchability was reduced with increased concentrations of *Annona reticulata* seed extract. Various authors have reported the egg hatchability is dose dependent, with hatchability decreasing with increasing concentrations of extracts[15]. According to Smith and Salkeld[16] differences in susceptibility of eggs to ovicides are due to differential rate of uptake, penetration through chorion, conversion to active inhibitor, detoxication and failure of the toxicant to reach the target.

Larvicidal and pupicidal effect of *Annona reticulata* seed extract against *Aedes aegypti*.

The I, III, IV th instar larvae and pupae of *Aedes aegypti* were exposed to 0.5 %,1.0 %,1.5 %, 2.0 % and 2.5% ethanol extract of *Annona reticulata* and the results are expressed in the table 2-5. LC_{50} values acetone seed extract for I, III, IV instar larvae and pupae of *Aedes aegypti* were 0.717 %, 1.263 %, 1.369 %, 1.425 % respectively (Table 3).

Among the various results obtained with ethanol seed extract of *Annona reticulata* acting on the I, III, IV instar larvae and pupae of *Aedes aegypti* at 24 hours exposure the I and III instar larvae showed maximum activity, with LC50 values of 0.717 % and 1.263 % respectively. In the present study, I instar larvae showed least susceptibility in *Aedes aegypti* with ethanol seed extract of *Annona reticulata.* Similar type of report was observed in the studies reported by Shyamala[17], Murugan[18], Vineetha and Murugan[19.] From the overall results, it was interesting to note that I instar larvae were more susceptible than the III and IV instar larvae and pupae. All these values are statistically significance at 5% level. Similarly the various solvent leaf extract of *Gloriosa superba* possessed larvidicidal and pupicidal activity against I, III, IV instar larvae and pupae of *Aedes aegypti* and *Culex quinquefasciatus*[20].

It was clear that, all concentrations eventually produced a high kill at a characteristic point in larval stage, whereas the lower dose treatments give more dispersed actions. It caused harm to larvae and pupae during moulting especially at the time of metamorphosis. This strongly suggested that the action was a hormone mimic. However from the experiments it is difficult to speculate on the actual mode of action of the plant extract. All the high dose, death occurred most in the larval stage.

CONCLUSION:

Our findings indicate that the toxic components responsible for larvicidal effect in the plant are concentrated in the ethanol extract of seeds. Results from our study revealed that the larvicidal

potential of *Annona reticulata* extract is compareable to previous studies on natural products. It is therefore suggested that the seed extract of *Annona reticulata* can be well utilised for preparing biocide and may serve as suitable alternative to synthetic insecticides as they are relatively safe, inexpensive and readily available in many areas of the world.

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