

## Larvicidal Efficacy of *Annona squamosa* Plant Extracts Against *Culex quinquefasciatus* Mosquito Larvae.



### Botany

KEYWORDS : • *Annona squamosa*, Methanol, *Culex quinquefasciatus*.

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### ABSTRACT

*Mosquitoes are associated with the transmission of malaria, dengue, chikungunya, japanese encephalitis, filariasis and other viral diseases throughout the globe. Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases. The present study was carried out to establish the properties of leaf extract of Annona squamosa was tested for larvicidal activity against Culex quinquefasciatus mosquito. The extracts of Annona squamosa commonly available and growing in India were tested for larvicidal activity against the 4th instar larvae of Culex quinquefasciatus using standard WHO technique. The lethality varied in plant methanolic extracts of Annona squamosa was found most effective and 100% result was observed in this treatment.*

### INTRODUCTION:

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Ethno botany is recognized as an effective way to discover future medicines. Plants may be an alternative source of mosquito-control agents because they constitute a rich source of bioactive chemicals (Sukumar *et al*, 1991; Wink, 1993). They are not only effective, but also greatly reduce the risk of potentially adverse ecological effects; they may prevent the possibility of the resistance that synthetic chemical insecticides typically bring about after prolonged use (Monzon *et al*, 1994).

Medicinal plants are commonly found throughout tropical and subtropical countries. A lot of research work on medicinal plants has been carried out on agricultural pests with promising results, and some have been produced at industrial level. However, very few have been reported for control of mosquito vectors.

Mosquito-borne diseases remain a major problem in the world, particularly in tropical and subtropical regions. These diseases contribute significantly to disease burden, death, poverty, and social debility in tropical countries (Young-Su *et al*, 2002). Although vector control programs have been established for a long time, the main method for control of the vectors is the use of chemical insecticides. However, the effectiveness of vector control has declined due to the development of resistance in mosquitoes against currently used insecticides (Chandre *et al*, 1998; Penilla *et al*, 1998). There are many serious drawbacks with the use of chemical insecticides, for example, the increasing cost of new insecticides and annual importation expenditures, effects on non-target populations especially on humans, environmental pollution, such as entering the food chain, and the development of insecticide resistance and the emergence of refractory vector behavior. One approach to this problem has been to search for new and effective compounds that do not have any ill effects on non-target populations, are easily degradable, safe, and easily available at low cost.

Some crude extracts from medicinal plants are currently being used as they are potentially economical, safe, and practical for control measures. Being "environmentally friendly," it is more practical and safe for the environment to use botanical insecticides to control mosquitoes. Therefore, this study was designed to focus on the *Annona squamosa* plant and their potential specific effects on *Cx. quinquefasciatus*.

### MATERIALS AND METHODS:



#### Collection and processing of medicinal plants:

The fresh and healthy leaves of *Annona squamosa* were collected from nagonda district, Telangana State, India. and washed with fresh water and shade dried at room temperature. The dried material was powdered and extracted with methanol for 3 days and filtered with watman filter paper 1. The extracts were concentrated at reduced temperature on a rotary evaporator and stored at 4°C.

#### Preparation of stock solution:

1 gram of crude extract was first dissolved in 100 ml of methanol and stored as stock solution. The anthelmintic assay was carried as per the method of Tandon *et al.*, 1997.

#### Selection of mosquito species:

The important vector species of mosquito borne which is *Culex quinquefasciatus* was selected for the present study. This species is the vector of west Nile virus which causes encephalitis or lymphatic filariasis or meningitis which is known to effect the brain tissue, finally resulting in permanent neurological damage (Hubble and Halouzka, 1999).

#### Collection and culture of mosquito larvae:

*Culex quinquefasciatus* larvae were collected from stagnant sewage water near Hyderabad, the collected larvae were reared from egg to larval stage and then to adults in the laboratory itself, to avoid the species mixture next fl generation larvae were used for the present study. This procedure facilitates to maintain the uniform age of larval instar. The 4<sup>th</sup> instar larvae were used to

test the various concentrations of extracts.

#### BIOASSAY:

The larvicidal activity was assessed by the standard guidelines of World Health Organization(2005), with some modification and as per the method of Rahuman *et al.*, (2000), Prabakar and Jebanesan (2004). A laboratory colony of *C. quinquefasciatus* was used for the larvicidal activity studies.

For bioassay test, twenty numbers of fourth instars larvae were taken in six batches for the treatment. Bowls of 100 ml capacity were kept in series, and tested for each desired plant extract concentrations 5, 10, 25, 50, 100 and 200 ppm. The control was set up with methanol and distilled water. The experimental media, in which 100% mortality rate of larvae occurred were selected for a dose response bioassay. Based on the screening results, the numbers of dead larvae were counted after every 12 hrs of treatment up to 48 hours. The percentage mortality and standard error of mean have been calculated for all the results obtained by this study.

#### Results and Discussions:

**Table 1:** Mortality rates of *Culex quinquefasciatus* mosquito larvae at different concentrations of crude extract of *Annona squamosa* leaves.

Conce. (ppm)	Time (hours)				n = 20			
	12	24	36	48	Total	%	Mean	SE (+/-)
5	5	10	12	16	16	80	4.000	2.287
10	7	11	14	17	17	85	4.250	2.136
25	8	13	16	18	18	90	4.500	2.175
50	11	16	18	20	20	100	5.000	1.931
100	13	18	20	-	20	100	6.660	1.652
200	20	-	-	-	20	100	20.000	0.000
Control	0	0	0	0	0	0	0.000	0.000

*n* = number of larvae used for bioassay test.

**Graph 1:** Mortality rates of *Culex quinquefasciatus* mosquito larvae at different concentrations of crude extract of *Annona squamosa* leaves.

The larvicidal activity of *A. squamosa* leaf crude extract at different concentration is represented in Table: 1. the data shows that, 100 % mortality rate of larvae was observed at 50, 100 and 200 ppm concentrations of crude extracts. At the concentration of 200 ppm 100 % mortality rate was recorded after 12 hrs, and at 100 ppm 100 % mortality rate was seen after 36 hrs. At low concentrations of crude extract i.e. 25, 10 and 5 ppm after 48 hrs of treatment the % mortality rate of mosquito larvae was decreased to 90, 85 and 80 % respectively. The percentage mortality and

standard error of mean have been calculated for all the results obtained by this study.

Das NG *et al.*, (2007) observed methanol leaf extract of *A. squamosa*, against *Cx. quinquefasciatus* mosquito larvae ranged between 31.80 and 155ppm. The variations found in the bioassay may be assigned to differences in the quality and quantity of active constituents of the crude extracts and also due to ecological conditions under which the plants were grown. Bhagavan *et al.*, (2009) of Tamilnadu observed 100 % mortality rate of *Culex tritaeniorhynchus* larvae when 106.41 ppm of *A. squamosa* leaves methanolic extract was used. He reported 100 % mortality rate of *Anopheles subpictus* larvae when 17.47 ppm of *A. squamosa* leaves Ethyl acetate extract was used. Joseph J. Magadula *et al.*, (2009) of Tanzania reported that ethanol extract of *A. Squamosa* was most active and showed 100 % mortality at 50 µg / ml on the *Cx. quinquefasciatus* mosquito larvae of late third instar.

In the previous research late 3<sup>rd</sup> instar or early 4<sup>th</sup> instar larvae of three different species of mosquito larvae were tested for 100 % mortality with different concentrations of *Annona squamosa* methanolic leaf extract, whereas in this study 4<sup>th</sup> instar larvae of *Cx. quinquefasciatus* showed more or less similar results.

#### CONCLUSIONS:

The present investigations have important implications in the practical control of mosquito larvae in the polluted aquatic ecosystem. The *Annona squamosa* plant is available in large quantities and can be used as environment friendly and sustainable insecticides to control mosquito. These extracts are easy to handle, inexpensive and safe natural products for mosquito control (Kalyanasundaram and Babu, 1982).

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