COMPARATIVE EVALUATION STUDY OF INSECTICIDAL PLANTS FOR THE CONTROL OF VECTOR MOSQUITO *ANOPHELES STEPHENSI*

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ABSTRACT

Objective: - Aim of this work was to comparative study on larvicidal activity of insecticidal plants against malaria spreading mosquito *Anopheles stephensi.*

Methods: - The larvicidal activity of Petroleum ether and Chloroform extracts of *Lantana camara* Linn.(whole plant) and *Toddalia asiatica* Lam. (whole plant) were tested against mosquito larvae of *Anopheles stephensi*. Late third or early fourth instar larvae were used for the screening. These extracts were used for determining the larvicidal activity by using WHO method for evaluation of new larvicidal agents.

Result: - The petroleum ether and chloroform extracts of *Lantana camara* showed highest larvicidal activity against the mosquito vector *Anopheles stephensi* in comparison to *Toddalia asiatica*. No mortality was observed in control.

Conclusion: - The result suggests the use of the plants in insect control as an alternative method for minimizing the noxious effect of some pesticide compounds on the environment. Thus the extracts of whole plant of *Lantana camara* Linn. and *Toddalia asiatica* Lam. may deliver promising more selective and biodegradable agents.

INTRODUCTION:

Mosquitoes are one of the most medicinally significant vectors and they transmit parasites and pathogens, which continue to have devastating impact on human beings.[1] Several numbers of species belonging to genera *Anopheles, Culex, Aedes* and vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, Dengue, and yellow fever. Thus one of approaches for control of these mosquitoes borne diseases is the interruption of disease transmission by killing or preventing mosquito bites.[2] Herbal

products which proven potential as insecticides or replicants can play an important role in the interruption of the transmission of mosquitoes borne diseases both at the individual and community level. However the discovery, development and use of synthetic organic insecticidal chemicals with persistent residual action not only over shadowed the use of herbal products as insecticides of choice against mosquitoes but also become the major weapon for mosquito control. [3]

But the extensive use of synthetic organic insecticides during the last decades has resulted in environmental hazards and also in the development of physiological resistance in most vector species. This has necessitated the need for research and development of environmentally safe, biodegradable, low cost indigenous methods for vector control, which can be use with minimum care by individual and communities in specific situations.[4]

The plant *Lantana camara* Linn. (Verbenaceae) and *Toddalia asiatica* Lam. (Rutaceae) are described in Ayurveda and Siddha, as a potent drug against a variety of ailments. These plants are widely distributed and cultivated in various parts of India. [5,6,7,8]

MATERIAL AND METHODS:

COLLECTION OF PLANT MATERIAL:

The plants were collected during flowering stage in the month of July-August from Nilgiris. Then their identification was established with the aid of an expertise botanist Dr. S. Rajan and compared with herbarium sheets of the authentic sample.

Many of defensive components are biodegradable with non-residual effect on the biological environment hence; an attempt has been made in present investigation to identify plants with potential to control vector mosquitoes.

EXTRACTION:

The plant *Lantana camara* Linn. and *Toddalia asiatica* Lam. were powdered and extracted in Soxhlet with Petroleum ether and Chloroform. The extracts were concentrated under reduced pressure to a semisolid mass. These extracts were used for determining the larvicidal activity against mosquito larvae.

BIOLOGICAL ASSAY:

Larvicidal activity was evaluated in accordance to WHO for the evaluation of new larvicidal agents.[9] The larvae of *Anopheles stephensi* was obtained and reared from the neonates in National Institute of Communicable Diseases, Southern India branch field station located at Mettupalayam (District Coimbatore of Tamil Nadu State), at 28 ± 2ºC with a photoperiod of 12 hours light and dark and 80± 10% RH. A brewer's yeast powder mixed with an equal quantity (w/w) of ground dog biscuit is used in laboratory as a food for larvae. The late third or early fourth instar larvae were collected according to larval size and degree of chitinization of respiratory siphon.[10] Different concentrations of the extracts were prepared in 1ml of acetone for each experiment. All experimental exposure was done in 500ml glass beaker in triplicate. 25 larvae were collected with a pasture pipette, placed on a filter paper for removal of excess of water and placed in 250ml dechlorinated tap

water containing various concentration of crude extracts. Three controls in triplicate were setup, one with acetone (1ml), the other with distilled water (250ml).

The beakers were covered with muslin cloth to avoid to entry of any foreign material. Sufficient control was also kept for each extracts. The observed mortality (Crude mortality) was recorded at 24 hours of exposure to test solution. From this crude mortality, percentage crude mortality was obtained. Subsequently control mortality if any was recorded and percentage crude mortality was obtained. The percentage crude mortality was corrected by using Abbot's formula. The corrected probit mortality and expected mortality was also obtained. But no control mortality recorded during the experiment so we have not used of Abbot's formula.

STATISTICAL ANALYSIS

LC_{50} and LC_{90} values and their 95% confidence limits were estimated by fitting a probit regression model to the observed relationship between percentage mortality of larvae and logarithmic concentration of the substance. Separate probit models were fitted for each extract.[11] All analysis was carried out using the (Statistical Package Social Science) SPSS software, version 13.0.

RESULT AND DISCUSSION:

Seven different concentrations of test solution ranging from 50-350 ppm for petroleum ether extract and six concentrations of test solution ranging from 50-300 ppm for chloroform extract for *Lantana camara* Linn. and nine different concentrations of test

solution ranging from 50-450 ppm for both petroleum ether extract and chloroform extract for *Toddalia asiatica* Lam. were subjected to 24 hr bioassay using early 4th instar larvae of *Anopheles stephensi.* Based on observations made in the 24 hours bioassay studies among the different plant extracts, the petroleum ether extract of *Lantana camara* Linn. was more potent than petroleum ether extract of *Toddalia asiatica* Lam. and chloroform extract of *Lantana camara* Linn. was more potent than *Toddalia asiatica* Lam. for vector mosquito and were identified as efficient against them.

The results from the *Anopheles stephensi* larvicidal bioassay using different extracts of two different plants the most active extract against late third or early fourth instar larvae of *Anopheles stephensi* were the petroleum ether and chloroform extract of *Lantana camara*. The results of susceptibility of larvae for the extracts were given in table I and II.

In conclusion the use of the plants in insect control offers a safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost. Moreover, these results could be useful in the search for newer, more selective and biodegradable larvicidal natural compounds.

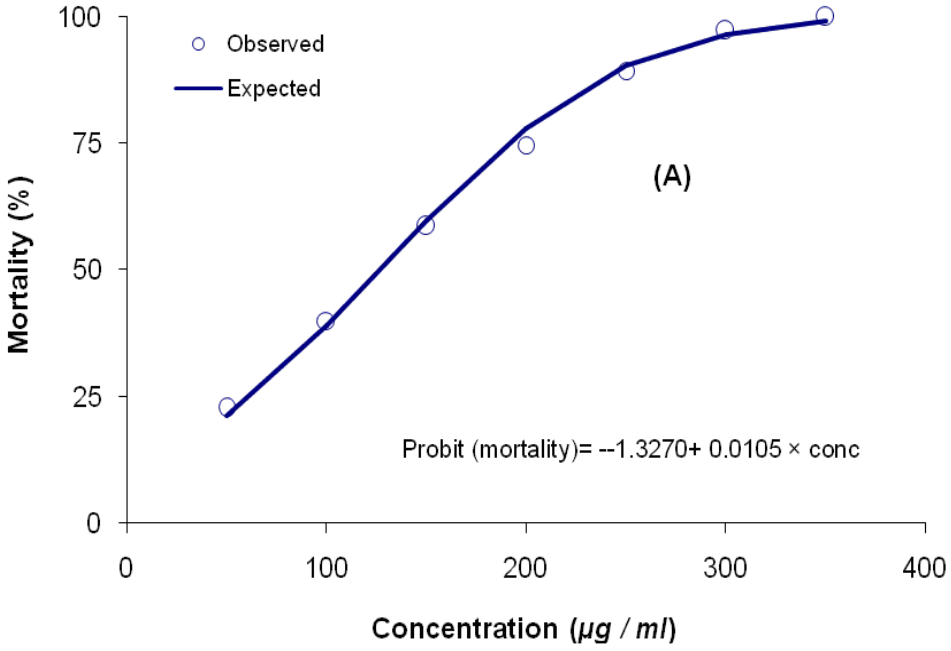
Table 1- Showing Observed and expected mortality of *Anopheles stephensi* larvae exposed to *Lantana camara* with

Conc. (µg/ml)	No. of Larvae		Mortality (%)		Expected Mortality			Probit (mortality) = a + b x conc	χ^2 D.F.P Value	LC_{50} (95 %CI)	LC_{90} (95 % CI)
	Exposed	Dead	Crude	Corrected	Probit	Dead	%				
Petroleum extract											
50	75	17	22.7	22.7	-0.80	15.8	21.1				
100	75	30	40.0	40.0	-0.28	29.3	39.1				
150	75	44	58.7	58.7	0.25	44.8	59.8				
200	75	56	74.7	74.7	0.77	58.5	78.0				
250	75	67	89.3	89.3	1.30	67.7	90.3	-1.3270+ 0.0105	χ^2 = 1.56	126.7	248.9
300	75	73	97.3	97.3	1.82	72.4	96.6				
350	75	75	100.0	100.0	2.35	74.3	99.1	x conc.	D.F.=5	(112.0-1	(231.2-27
									P = 0.9	39.7)	1.8)
Chloroform extract											
50	75	8	10.7	10.7	-1.95	1.9	2.6				
100	75	9	12.0	12.0	-1.16	9.2	12.3				
150	75	13	17.3	17.3	-0.37	26.7	35.5	-2.7408+ 0.0158	χ^2 = 39.9	173.8	255.0
200	75	44	58.7	58.7	0.42	49.7	66.2	x conc.	D.F.=4	(120.2-2	(205.5-43
250	75	73	97.3	97.3	1.21	66.5	88.7				
300	75	75	100.0	100.0	2.00	73.3	97.7		P <0.005	35.3)	5.0)

Petroleum ether and Chloroform extracts. Expected mortality is based on probit regression analysis.

D.F. = Degrees of freedom

Figure 1- Relation between *Anopheles stephensi* larval mortality and concentration of *Lantana camara* with (A) Petroleum ether and (B) Chloroform extracts. Expected values are based on probit regression analysis.



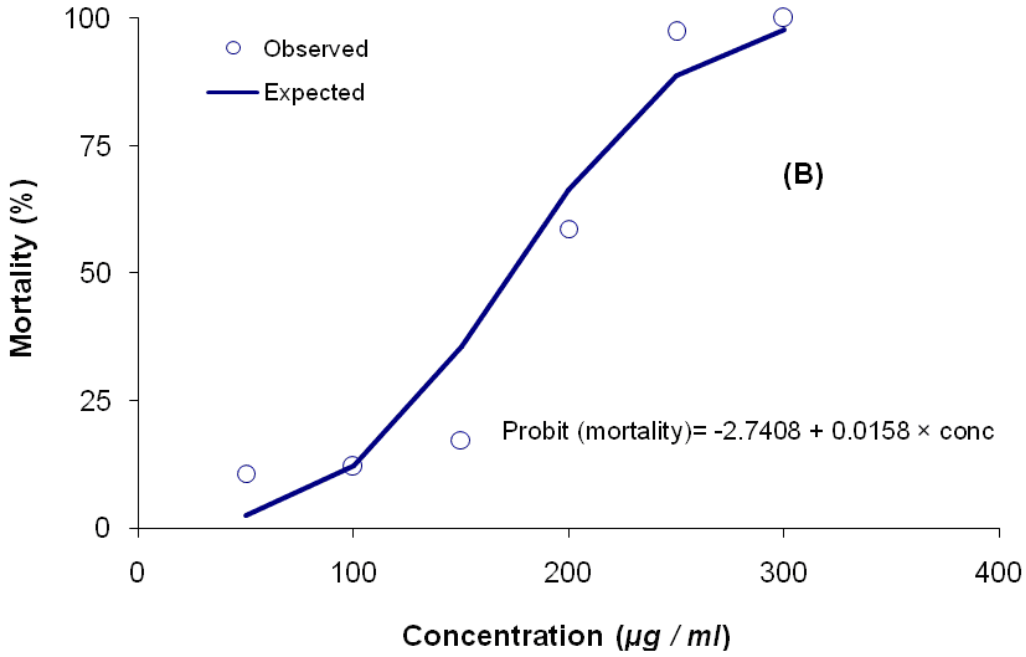

100
75
50
25
0
Mortality (%)
Observed
Expected
(B)
Probit (mortality)= -2.7408 + 0.0158 × conc
0
100
200
300
400
Concentration (µg / ml)

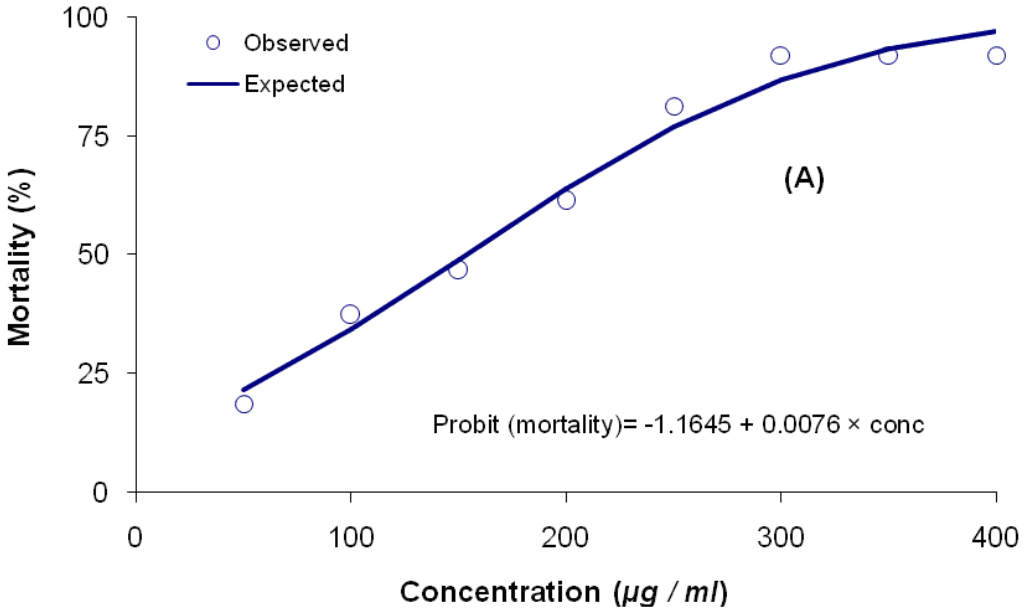
Table 2- Observed and expected mortality of *Anopheles stephensi* larvae exposed to *Toddalia asiatica* with Petroleum ether and Chloroform extracts. Expected mortality is based on probit regression analysis.

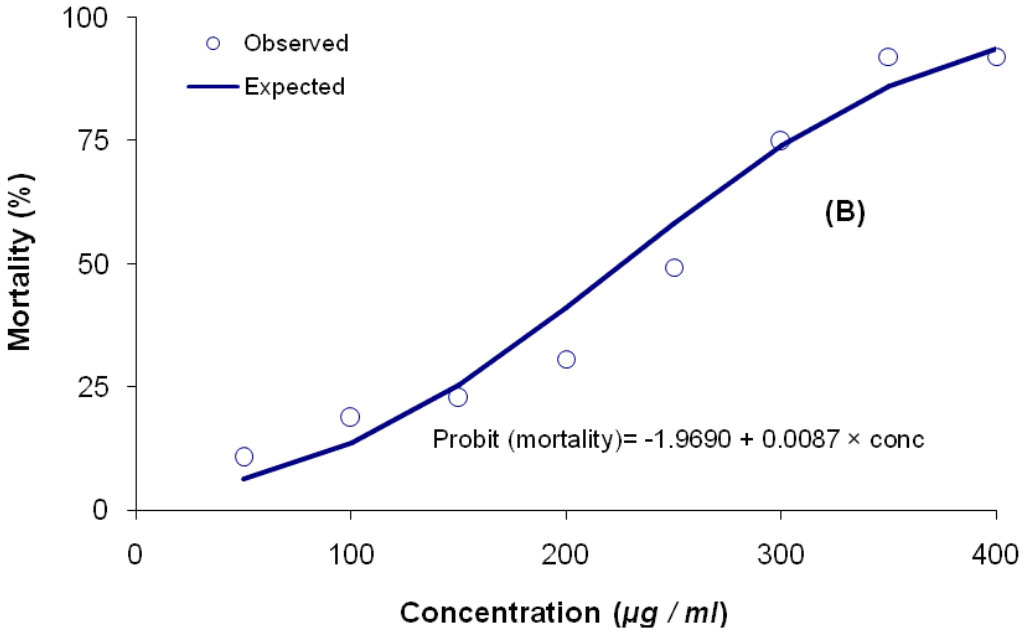
Conc. (µg/ml)	No. of Larvae		Mortality (%)		Expected Mortality			Probit (mortality) = a + b x conc.	χ^2 D.F P Value	LC_{50} (95 %CI)	LC_{90} (95 % CI)
	Exposed	Dead	Crude	Corrected	Probit	Dead	%				
Petroleum ether extract											
50	75	14	18.7	18.7	-0.78	16.2	21.6	-1.1645+ 0.0076 x conc.	χ^2 = 10.9 D.F.=7 P=0.14	153.7 (135.8-169.6)	322.8 (301.5-349.4)
100	75	28	37.3	37.3	-0.40	25.7	34.3				
150	75	35	46.7	46.7	-0.02	36.8	49.0				
200	75	46	61.3	61.3	0.36	47.9	63.9				
250	75	61	81.3	81.3	0.74	57.7	76.9				
300	75	69	92	92	1.12	65.1	86.8				
350	75	69	92	92	1.50	69.9	93.3				
400	75	69	92	92	1.88	72.7	97.0				
450	75	75	100	100	2.26	74.1	98.8				
Chloroform extract											

50	75	8	10.7	10.7	-1.53	4.7	6.3	-1.9690+ 0.0087 x conc.	χ^2= 14.6 D.F.=7 P =0.04	226.6 (202.5-250.2)	374.1 (340.6-421.9)
100	75	14	18.7	18.7	-1.10	10.2	13.6				
150	75	17	22.7	22.7	-0.66	19.0	25.3				
200	75	23	30.7	30.7	-0.23	30.7	40.9				
250	75	37	49.3	49.3	0.21	43.6	58.2				
300	75	56	74.7	74.7	0.64	55.4	73.9				
350	75	69	92	92	1.08	64.4	85.9				
400	75	69	92	92	1.51	70.1	93.5				
450	75	75	100	100	1.95	73.1	97.4				

D.F. = Degrees of freedom

Figure 2- Relation between *Anopheles stephensi* larval mortality and concentration of *Toddalia asiatica* with (A) Petroleum ether and (B) Chloroform extracts. Expected values are based on probit regression analysis.





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