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Larvicidal and Pupicidal Activity of *Terminalia catappa* Leaf Extracts on *Aedes aegypti* Mosquito: A Vector Intervention

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Abstract: *The effect of aqueous, ethanol and acetone extracts of Terminalia catappa leaves against the larvae and pupae of Aedes aegypti mosquito was studied. Early 3rd instar larvae of Aedes aegypti mosquitoes and pupae were exposed for up to three days, to a dilution of 2, 4, 6, 8 and 10% of aqueous extracts and 100, 200, 300, 400 and 500ppm of ethanol and acetone extract of leaves. All tested extracts showed larval mortality. Except aqueous extract, other extracts showed pupal mortality. However, larval mortality was greatest with the ethanol extract followed by acetone and aqueous extract. Maximum pupal mortality was observed in acetone extract followed by ethanolic extract. The aqueous extract did not show any pupal mortality. Based on Probit analysis, the* LC_{50} *values of aqueous, ethanol and acetone extract of Terminalia catappa for the 3rd instar larvae was found to be 5%, 166.0ppm and 177.8 ppm and for the pupae it was 169.8 ppm and 161.4 ppm for ethanolic and acetone extract respectively. The results were statistically significant at* $P<0.05$ *level. Qualitative analysis of the extracts revealed the presence of saponins, tannins, flavonoids, alkaloids, terepenoids and phenols. The results suggest that the leaf extract of Terminalia catappa could be used as an effective herbal larvicidal and pupicidal agent against Aedes aegypti without causing damage to the environment*

Keywords: *Aedes aegypti , Terminalia catappa, LC50, mortality, pupae, larvae*

I. Introduction

Mosquitoes are one of the most medically significant vectors, and they transmit parasites and pathogens, which continue to have devastating impact on human beings. The vector borne diseases caused by mosquitoes are one of the major health problems in many countries. Malaria, dengue, yellow fever, filariasis, and chickungunya are some of the deadly diseases spread by mosquitoes (Maheswaran, *et al*., 2008). *Aedes aegypti* is the principal vector of dengue fever and dengue hemorrhagic fever and it is reported to infect more than 100 million people every year in more than 110 countries in the tropics. Thus one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes to bite human beings (Rajmohan, *et al*.). Herbal products with proven potential as insecticide or repellent can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level. But the development and use of synthetic organic mosquito repellents have over shadowed the use of herbal products against mosquitoes and have become the major weapon for mosquito control. Since the discovery of DDT, mosquito control approach has been almost completely based on synthetic organic insecticides. But the extensive use of synthetic organic insecticides during the last five decades has resulted in environmental hazards and also in the development of physiological resistance in major vector species. This has necessitated the need for search and development of environmentally safe, biodegradable, low cost, indigenous methods for vector control, which can be used with minimum care by individual and communities in specific situations.

Phytochemicals obtained from plants with proven mosquito control potential can be used as an alternative to synthetic insecticides or along with other insecticides under the integrated vector control. Toxicity of phytochemicals in mosquitoes was first reported by Campell et al. (1933). In a review paper , Sukumar et al (1991) summarized a list of 104 plant species for 49 families that possessed either larvicidal or pupicidal and/or adulticidal activity on Aedes aegypti. In 2006, Chaithong et al reported the larvicidal activity of ethanolic extract of Piper longum against Aedes aegypti. Samuel Tennyson etal in 2013 reported Larvicidal Efficacy of some Plant Oils Against the Dengue Vector Aedes aegypti.

II. Taxonomy of the plant

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Myrtales
Family	Combretaceae
Genus	Terminalia

Speceis catappa

Terminalia catappa is widely grown in tropical regions of the world as an ornamental tree, for the shade its large leaves provide. The fruit is edible, tasting slightly acidic. It grows to 35 m tall, with an upright, symmetrical crown and horizontal branches. As the tree gets older, its crown becomes more flattened to form a spreading, vase shape. The leaves are large, 15-25 cm long and 10-14 cm broad, ovoid, glossy dark green and leathery. They are dry-season deciduous; before falling, they turn pinkish-reddish or yellow-brown, due to pigments such as violoxanthin lustein, and zeaxanthin.

The leaves have many medicinal uses including diaphoretic, anti-indigestion, and anti-dysentery. Bark, leaves and unripe fruits are astringent haemostatic, digestive, antiseptic etc. Seeds are nutritive, sweet, bitter, astringent, etc. edible as substitute for real almond. *Terminalia catappa* is used to prepare ointment for scabies, leprosy and other skin diseases. An infusion of the young leaves or scraped bark is occasionally taken as a potion for treating mouth infections in Tonga and Samoa. Young leaves are used in the Philippines to cure headache and colic. The bark is astringent, mild diuretic, cardiotonic and antidysenteric. Leaf is sudorific, antirheumatic, antileprotic and anticephalalgic. Fish breeders use the leaves in an aquarium to lower the pH and heavy metal content of the water.

During the course of last two decades, many plant extracts have been evaluated for their larvicidal activities, in order to find a method of biological control of mosquitoes; very few studies were carried out to assess the the larvicidal effect of saponin-rich plants (Pelah et al., 2002). The objective of the study was to assess the efficacy of *Terminalia catappa* leaf extracts against the larvae and pupae of *Aedes aegypti* mosquito.

III. Materials and methods

Fresh leaves of *Terminalia catappa* were collected and identified by an expert taxonomist.The leaves were collected, washed, chopped and air dried in a shady place to retain their active ingredients intact. The dried materials were ground in a table model grinder to form a fine powder and kept in an air tight container for further use. The powdered leaves were used separately for the preparation of different extracts of different concentrations. Extraction was carried out using as simple a method as possible, thinking that it could be repeated any remote areas of the world where *Terminalia catappa* plants grow naturally. The idea is to allow for easy adoption of this methodology by the local communities. To prepare the aqueous extract, 30 grams of leaf powder was placed in separate conical flasks, 300ml of distilled water was added, and mixed vigorously. The mixture was kept for 24h on rotary shaker. After twenty four hours, the mixture was filtered using a Whatmann filter paper no. 1. Volume of extract was adjusted to 300ml using distilled water to make 10% stock solution. For ethanolic and acetone extract, thirty grams of leaf powder was placed in separate conical flasks, 300ml of ethanol (75%) and acetone was added respectively, and mixed vigorously. The mixture was kept for three days at room temperature. After three days, the mixture was filtered using a Whatmann filter paper no.1. The solvent was evaporated on boiling water bath. The residue obtained was dissolved in 75% ethanol and acetone respectively to make a stock solution of 1%.

IV. Qualitative and quantitative estimation of phytochemicals

Qualitative analysis of the aqueous, acetone and ethanolic extract of *Terminalia catappa* leaf was performed using standard procedures as described by Sofowara (1993), Trease and Evans (1989), and Harborne (1973).

Quantitative analysis of the extract for Total Phenol, Tannins, Saponins, Aalkaloid and Flavanoid was carried out using standard procedures suggested by Folin- Ciocalteau, Folin- Denis, Obadoni- Ochuke, Horborne's Method and Bohm and Kocipai Abyazan respectively.

V. Rearing of mosquito larvae

Larvae of *Aedes aegypti m*osquito were collected from HAFFKINE INSTITUTE, PAREL, MUMBAI. They were colonized and maintained continuously in the laboratory free of exposure to pathogens, insecticides or repellants. They were maintained at controlled conditions of 28^{0}C temperature and 70-80% relative humidity. Larvae were fed on finely ground dog biscuit. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages for adult emergence. The adults of *A. aegypti* were reared in the glass cage. The adult colony was provided with 10% sucrose solution in cotton pads and it was periodically blood-fed on restrained rats. The eggs of *A. aegypti* were obtained as egg rafts on a filter paper. The egg rafts of *A. aegypti* were kept in a tray containing tap water (as culture medium) at laboratory conditions (29 ± 1°C). On the next day, the eggs were observed to hatch out into first instar larvae. On the third day after hatching, the first instar larvae moulted into second instar larvae. On the fifth day, third instar larvae were observed which moulted into fourth instar larvae on the seventh day. The third instar larvae of *A. aegypti* were used for the present study.

VI. Treatment of larvae with leaf extract

For treatment of larvae with the leaf extract of *Terminalia catappa*, 100 ml of tap water was kept in a series of glass beakers (of 200 ml capacity). Required quantity of stock solution was added into each beaker (containing 100 ml of tap water) to obtain a particular concentration of the extract. Control for aqueous extract contained 100ml distilled water and for ethanol and acetone extract it contained 500ppm of ethanol and acetone respectively. The larval mortality of third instar larvae of *A. aegypti* was observed separately in control, 2, 4, 6, 8, 10% concentrations of aqueous extract and 100, 200, 300, 400, and 500 ppm concentrations of the ethanol and acetone extract of *Terminalia catappa*. Ten larvae of the early 3^{rd} instars were placed in were separately introduced into control and different concentrations of leaf extract. After adding the larvae, the beakers were covered with muslin cloth and were kept in the growth room maintained at room temperature. Experiments were done in triplicates. Based on the per cent mortality values, Lc50 value of leaf extract of *Terminalia catappa against A. aegypti* were obtained by calculating the regression line employing Probit analysis.

VII. Results and discussion

Table: 1

Qualitative and Quantitative Analysis of Phytoconstituents of *Terminalia catappa*

Phytochemicals	Qualitative Tests	Quantitative analysis (%) per gram dry leaf powder
Tannins	Present	12.43
Saponins	Present	28.03
Flavanoids	Present	10.7
Alkaloids	Present	10
Steroids	**Absent**	-
Phenols	Present	23.2
Terpenoids	Present	-
Cardiac Glycosides	Present	-

Table: 2

Mortality Range of aqueous extract of *Terminalia catappa* on 3^{rd} instar larvae of *Ae.aegypti*

Sr.No	Concentration (%)	Mean % mortality	S.D	S.E	Range at (95%) Confidence limit
1	Control	-	-	-	-
2	2	26.7	5.536	3.196	20.43- 32.97
3	4	30	10	5.774	18.68 – 41.32
4	6	43.3	6.136	3.542	36.36 – 50.24
5	8	46.7	5.353	3.090	40.64 – 52.76
6	10	63.3	6.297	3.636	56.17 – 70.43

Table: 3

Mortality Range of ethanolic extract of *Terminalia catappa* on 3^{rd} instar larvae of *Ae.aegypti*

Sr.No	Concentration (ppm)	Mean % mortality	S.D	S.E	Range at (95%) Confidence limit
1	Control	-	-	-	-
2	100	36.7	5.447	3.145	30.54- 42.86
3	200	43.3	6.136	3.543	36.36 – 50.24
4	300	93.3	6.531	3.771	85.9 – 100.7
5	400	100	-	-	-
6	500	100	-	-	-

Table: 4

Mortality Range of acetone extract of *Terminalia catappa* on 3^{rd} instar larvae of *Ae.aegypti*

Sr.No	Concentration (ppm)	Mean % mortality	S.D	S.E	Range at (95%) Confidence limit
1	Control	-	-	-	-
2	100	26.7	11.430	6.599	13.77 – 39.63
3	200	30	--	-	-
4	300	33.3	15.383	8.882	15.89 – 50.71
5	400	46.7	5.352	3.090	40.64 – 52.76
6	500	53.3	6.216	3.59	46.27 – 60.33

Table: 5

LC_{50} values , regression equation and t- values of aqueous, ethanol and acetone extract of *Terminalia catappa* for 3rd instar larvae of Ae.aegypti

Name of the Plant	Solvent used	LC_{50}	Regression Equation	Calculated t value	t value at (0.05)	t value at (0.01)
Terminalia catappa	Aqueous	5.9%	Y=2.504+ 3.236X	6.43***	2.78	4.60
	Ethanol	166.0ppm	Y=5.786+ 4.934X	5.25***	2.78	4.60
	Acetone	177.8ppm	Y=2.833+ 3.106X	7.44***	2.78	4.60

Table: 6

Mortality Range of Aqueous extract of *Terminalia catappa* on pupae of *Ae.aegypti*

Sr.No	Concentration (%)	Mean % mortality	S.D	S.E	Range at (95%) Confidence limit
1	Control	0	-	-	-
2	2	0	-	-	-
3	4	0	-	-	-
4	6	0	-	-	-
5	8	0	-	-	-
6	10	0	-	-	-

Table: 7

Mortality Range of Ethanolic extract of *Terminalia catappa* on pupae

Sr.No	Concentration (ppm)	Mean % mortality	S.D	S.E	Range at (95%) Confidence limit
1	Control	-	-	-	-
2	100	36.7	11.39	6.57	23.82-49.58
3	200	36.7	5.445	3.14	30.55-42.85
4	300	46.6	11.94	6.9	33.08-60.12
5	400	93.3	6.531	3.77	85.91-100.69
6	500	100	-	-	-

Table: 8

Mortality Range of Acetone extract of *Terminalia catappa* on pupae of *Ae.aegypti*

Sr.No	Concentration (ppm)	Mean % Mortality	S.D	S.E	Range at (95%) Confidence limit
1	Control		-	-	-
2	100	33.3	6.053	3.495	26.45-40.15
3	200	83.3	6.453	3.726	75.99-90.60
4	300	100	-	-	-
5	400	100	-	-	-
6	500	100	-	-	-

Table: 9

LC_{50} values ,regression equation and t- values of aqueous, ethanol and acetone extract of *Terminalia catappa* for pupae of Ae.aegypti

Name of the Plant	Solvent used	LC_{50}	Regression Equation	Calculated t value	Table t value at (0.05)	Table t value at (0.01)
Terminalia catappa	Aqueous	-	-			
	Ethanol	169.8ppm	Y=5.803+4.726X	4.62***	2.78	4.60
	Acetone	161.4ppm	Y=4.227+4.436X	6.46***	2.78	4.60

The qualitative analysis of aqueous extract of *Terminala catappa* revealed the presence of tannins, saponins, flavonoids, terpenoids, alkaloids, phenols and cardiac glycosides. Crude saponin was the major constituent (28.03%) followed by phenols (23.2%) and tannins (13.6%) in *Terminalia catappa*. Other phytochemicals estimated are present in low concentration**.**

The percent mortality values of 3rd instar larvae treated with different concentration (ranging from 2 to 10% for aqueous and 100 to 500ppm for ethanol and acetone) and 95% upper and lower fiducial limits of the leaf extract of *Terminalia catappa* at the end of 72hrs are represented in Table 2-4. The LC_{50} values, regression equations and the t- values of the leaf extracts of *Terminalia catappa* for 72h of exposure of *A. aegypti* are given in Table 5.

Based on the Probit analysis, the LC_{50} value of the aqueous, ethanolic and acetone extract of *T. catappa* was found to be 5.9%, 166.0ppm.and 177.8ppm.respectively. The results showed that the larvicidal activity of aqueous, ethanol and acetone extract of *Terminalia catappa* against *Ae. Aegypti* larvae varied according to the nature of extract. The ethanol extract of *Terminalia catappa* was found to be most effective.

The effect of each of the three extracts of *Terminalia catappa* (aqueous, ethanol and acetone) was found to be highly significant.

The percent mortality values of pupae treated with different concentration (ranging from 2 to 10% for aqueous and 100 to 500ppm for ethanol and acetone) and 95% upper and lower fiducial limits of the leaf extract of *Terminalia catappa* at the end of 72hrs are represented in Table 6-8. The LC_{50} values, regression equations and the t- values of the leaf extracts of *T.catappa* for 72h of exposure of *A. aegypti* are given in Table 9.

Based on the Probit analysis, the LC_{50} value of the ethanolic and acetone extract of *T. catappa* was found to be, 169.8ppm.and 161.4ppm.respectively. The effect of each of the two extracts of *Terminalia catappa* (ethanol and acetone) was found to be highly significant.

Nowadays, mosquito control is mostly directed against larvae and only against adults when necessary. This is because the fight against adult is temporary, unsatisfactory and polluting for the environment, while larval treatment is more localized in time and space resulting in less dangerous outcomes. Larval control can be an effective control tool due to the low mobility of larval mosquitoes, especially where the principal breeding habitats are manmade and can be easily identified. The secondary compounds of plants make up a vast repository of compounds with a wide range of biological activities. The larvicidal activity of many of the secondary compounds, such as saponins (Hostettmann and Marston *1995*; Wiesman and Chapagain*2005*; Pelah*et al.2002*), phenolics (Marston et al. *1993*; David et al. *2000*), isoflavonoids (Joseph et al. *2004*), essential oil (Carvalho et al. *2003*; Sukumar et al. *1991*), alkaloids (Lee, *2000*; Francois et al. *1996*), and tannin compounds (Khanna and Kannabiran*2007*), were well documented

The present study also revealed that probably one of the secondary compounds or a combination of few of them are responsible for the larval and pupal mortality. However, from the present study, the detailed structure of the active principle or the combinations of active principles responsible for larval mortality cannot be ascertained, and further study is necessary in this respect.

VIII. Conclusion

Terminalia catappa can be a potential candidate against *Aedes aegypti* particularly in its larvicidal and pupicidal activity. Ethanol extract of the dried leaf of the plant showed maximum larvicidal activity and acetone extract showed maximum pupicidal activity. As this plant is distributed throughout the country, the larvicidal and pupicidal properties of this plant species can be utilized while planning alternative vector control strategies based on integrated vector control measures through community based approaches. The plants are easily available to the local people and being an ayurvedic herb with multiple medicinal properties, it may be easily acceptable to them, since during application it would neither cause any toxic effect nor any additional burden. However, further studies on the identification of the active principles involved and their mode of action and field trials are needed to recommend *Teminalia catappa* as an anti-mosquito product used to combat and protect from mosquitoes in a control program.

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References

[1] Maheswaran,R., Sathish,S., Ignacimuthu,S. Larvicidal activity of *Leucasaspera*against the larvae of *Culexquinquefasciatus* Say.And *Aedesaegypti*L. *International J of Integrative Biology, 2,* 2008.

[2] Campbell. F.L, Sullivan. W.W, Smith L.N., The relative toxicity of nicotine, nabasine, methylanaba sine and lupinine for Culicine mosquito larvae, *J. Econo. Entomol., 26*, 1993, 505-509

[3] Raj Mohan, Ramaswamy, M, Evaluation of larvicidal activity of the leaf extract of a weed plant, *Ageratinaadenophora*, against two important species of mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*. *African Journal of Biotechnology,6 ,2007*, 631-638.

[4] Sukumar K, Perich M. J, Boobar L. R, Botanical derivatives in mosquito control: A review. *J Amer Mosquito Control Associatio, 7,* 1991, 210-237.

[5] Chaithong, U. Choochote, W. Kamsuk, K. Jitpakdi, A .Tippawangkosol P, Chaiyasit D, Champakaew D, Tuetun B, Pitasawat B., Larvicidal effect of pepper plants on Aedes aegypti (L.) (Diptera: Culicidae, J Vector Ecol. Jun, 31(1), 2006, 138-44.

[6] Dr. Samuel Tennyson., Samuel Tennyson, D. Arul Samraj, D. Jeyasundar , Keduokhrienuo Chalieu., Larvicidal Efficacy of Plant Oils Against the Dengue Vector Aedes aegypti (L.) (Diptera: Culicidae), *IDOSI Publications*, 2013.

[8] Sofowora, A. Medicinal plants and Traditional Medicine in Africa. *Spectrum Books Ltd (Pub.), Ibadan.*, 1993 .

[9] Trease, G.E., Evans, W.C. , Pharmacognosy. Brailliar Tiridel can, *Macmillian Publishers, 13*, 1989.

[10] Harborne, J.P, Phytochemical Methods: A guide to modern technique of plant analysis, *Chapman and Hall, London,2*, 1998.

[11] Hostettmann, K, Marston A, Saponins (chemistry and pharmacology of natural products). *University Press, Cambridge*, 1995, 132.

[12] Chapagain, B, Wiesman, Z, Larvicidal effects of aqueous extracts of *Balanite saegyptiaca*(desert date) against the larvae of *Culexpipiens*mosquitoes. *African Journal of Biotechnology, 4 ,11,* 1995, .1351-1354.

[13] Pelah D, Abramovich Z, Markus A, Wiesman Z , The use of commercial saponin from *Quillaja saponaria* bark as a larvicidal agent against *Aedes aegypti* and *Culex pipiens. J. Ethnopharm., 81*, 2002, 407-409.

[14] Marston. A., Maillard, M. and Hostettmann, K. Search for antifungal, Molluscicidal and larvicidal compounds from African medicinal plants. 1 Ethno-Pharmacol. Limerick: *Elsevier Scientific Publisher,s 38(2/3)* 1993, 215-223.

[15] David, J. P., Rey, D., Marigo, G., and Meyran, J. C, Larvicidal effect of a cell-wall fraction isolated from alder decaying leaves, *J. Chem. Ecol.* 26, 2000, 901–913.
[16] Joseph Howing Chan, PCT Int. Appl. WO 02 70470 (Cl. CO7C311/51) *Chem. Abstr, 137(6)*, 2004.
[17] Arvalho Carvalho, Melo V, Craveiro A, Machado M, Bantim M. B, Rabelo EF 2003. Larvicidal activity of the essential oil from Lippia sidoides Cham. against Aedes aegypti L. *Mem Inst Oswaldo Cruz* 98, 2003, 569-571.
[18] Sukumar K, Perich MJ, Boobar LR 1991. Botanical derivatives in mosquito control: a review. *J Am Mosq Control Assoc*, 2003, 210-237.
[19] Lee, Y.W, Zairi, J, Field evaluation of Bacillus thuringiensis H-14 against *Aedes mosquitoes, Tropical Biomedicine 23(1)*, 2006, 37–44.
[20] François G, Van Looveren M, Timperman G, Chimanuka B, Aké Assi L, Holenz J, Bringmann G, Larvicidal activity of the naphthylisoquinoline alkaloid dioncophylline A against the malaria vector *Anopheles stephensi. Nov, 54(2-3)*, 1996, 125-30.
[21] Khanna, V, Gopiesh, Kannabiran, K. Larvicidal effect of *Hemidesmusindicus*, *Gymnemasylvestre*, and *Ecliptaprostrata* against *Culexqinquifaciatus*mosquito larvae, *African Journal of Biotechnology, 6(3)*, 2007, 307-31.