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Larvicidal activity of *Ocimum sanctum* (Tulsi) against the dengue vector, Aedes aegypti

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ABSTRACT:

Mosquitoes are ectoparasites which transmit the diseases like as Malaria, dengue, Chikungunea, yellow fever, Filariasis, encephalitis etc. The yellow fever mosquito, Aedes aegypti is a mosquito that can spread the dengue fever, Chikungunea and yellow fever viruses, and other diseases. The mosquito can be recognized by white markings on legs and a marking in the form of a lyre on the thorax. Aedes aegypti is a vector for transmitting several tropical fevers. Only the female bites for blood which she needs to mature her eggs. Dengue fever also known as break bone fever is an infectious tropical disease caused by the dengue virus. Dengue is transmitted by several species of mosquito within the genus Aedes, principally A. aegypti

The mosquito larvae exposed to plant extract ***Ocimum sanctum*** *showed significant behavioral changes. The changes were observed within 1hr of exposure. The most obvious sign of behavioral change observed in Aedes aegypti larva incivility activity of larvae also showed restlessness loss equilibrium and finally led to death. These behavioral effects were more pronounced for Osmium sanctum extract after exposures. This effect may be due the presences of neurotoxin compound in this plants. For the control group such behavioral change was turbidity unit etc.*

KEYWORDS : *Turbidity, Larvae. Extract. LC_{50}.*

MATERIAL AND METHOD:

Conical flask, Burette, measuring cylinder, stand and test tubes, TLC jar, muslin cloth, beaker, warm water, egg strip, culturing of *Aedes aegypti* etc.

Chemicals: H_2SO_4, methyl orange, Phenolphthalein, universal indicator, stored water.

My research paper study is comprised of following four aspects

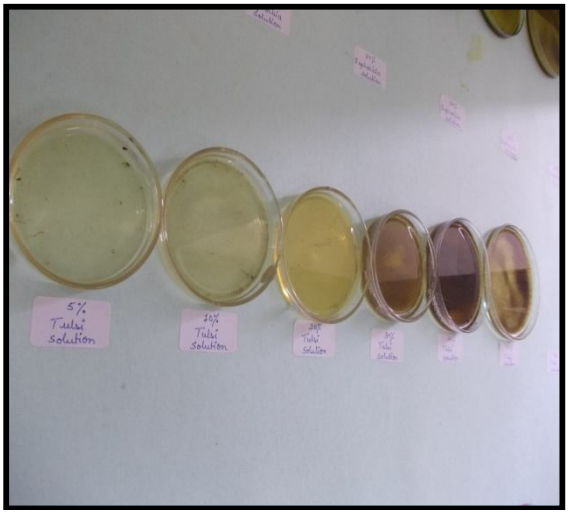
1) Physico-chemical analysis of water to be used for experiment
2) Culturing of *Aedes aegypti*.
3) Preparation of plant extracts of various concentration.
4) Study of Larvicidal activity of *Ocimum sanctum.*

I have taken a jar kept in warm water, put egg strips for two and three days, after four days eggs hatched and few larvae emerged out and again trial culturing of *Aedes aegypti*. I have taken large strip of egg about hundred and ten larvae emerged out. Feed with small pieces of biscuits and used for the experiment. I used to take precautions like **1**) Taken warm water. 2) Temperature is maintained at 37c. 3) Covering to the jar to avoid other species contamination.

Procedure: Fully developed leaves of the plants Tulsi (***Ocimum sanctum***) were collected during the flowering season of plant. From the Shade-dried leaves of the plant powder is made. We took 10gm powder of ***Ocimum sanctum*** then added 50ml methanol kept for whole night and next day added Dimethyl sulphoxide to it and used as stock solution for the further analysis. For the experiment group the extract was diluted with distilled water .The methanol and Dimethyl sulphoxide is used as the solvent.

Ocimum Sanctum extract (ml)	Distilled water
5ml extract	95ml D.W.
10ml extract	90ml D.W.
20ml extract	80ml D.W.
30ml extract	70ml D.W.
40ml extract	60ml D.W.
50mlextract	50mlD.W.

Photograph showing fresh dried leaves powder and extract *Ocimum Sanctum* (Tulsi)



Photograph showing the experimental & control group set exposed to *Ocimum sanctum* (Tulsi) plant extracts.

DISCUSSION:

The Physical and chemical properties of water used for the study are temperature, pH. The 24hrs bioassay is a major tool for evaluating the toxicity of phytotoxin and a number of researcher have been applying this method to assess the toxic effect of different extract against mosquito larvae. The mosquito larvae exposed to plant extract ***Ocimum sanctum*** showed significant behavioral changes. The changes were observed within one hour. of exposure. The most obvious sign of behavioral change observed in ***Aedes aegypti*** larva inhibitory activity of larvae also showed restlessness loss equilibrium and finally led to death. These behavioral effects were more pronounced for ***Ocimum sanctum*** extract after exposures. This effect may be due the presences of neurotoxin compound in both plants. For the control group such behavioral change was observed.

Therefore much effort has been put into the development of different insect repellants. Since synthetic agents often have severe toxic effects and may be too expensive for people in developing countries much hope has been placed on insect repellants of plant origin, preferably plants growing locally. ***Ocimum***species have been studied along with many others in this respect and the results of these studies are reported below. The essential oil (2% in acetone) of ***O.gratissimum***showed 100% repellency against the housefly, ***Musca domestica***(Singh and Singh 1991).Similar observations of Ocimum sanctum we have made for different species of insect ***Aedes aegypti*.**

Earlier the essential oil of *O.basilicum* showed repellent activity of class IV against red flour beetle, *Tribolium castaneum* (Mohiuddin *et al.,* 1987), but in contrast here we have used the leaf extract of Ocimum sanctum. Stein *et al* showed the effect, with a mortality rate of 79%, was observed for *M.dirhodum* (Stein *et al.,* 1988) but the observation which we noticed in the present study, with a mortality rate of 100%, for *Aedes aegyti*.

Satpathi and Ghatak scientist show that methanol extracts of leaves of *O.sanctum* at a concentration 1.0% resulted in 90%mortality of the grub of, a pest of brinjal, at 12 to24h after treatment. The observations were noticed in the present study the methanol extracts of leaves of *O.sanctum* at a concentration 50% resulted in 100%mortality.The Linn showed that Ocimum sanctum has larvicidal repellant properties against *Aedes aegypti* and for the neem seed extracts showed larvicidal activity on *Aedes aegypti* .

The similar observations were noticed in the present study and support the potential application of herbals plants in mosquito control measures; here we used ***Ocimum sanctum*** leaf extract.

The earlier research revealed the presence of phenolic compound in Leaf. They used Alcoholic extract in the Petri dishes inoculated with the cultures of different micro organisms, and these extracts were showed significant antimicrobial activity.. The antimicrobial screening shows a remarkable inhibition of growth of this micro organism.. This result indicates significant antibacterial activity observed by definite zone of inhibition produced by different zone of extracts. (S.R. Kawadikar1976)

A group of six mosquito larva having same weight, size, age were introduced into each Petri dish with various concentration of Tulsi and Euphorbia solution kept in Petri dish. The concentration ranging from 0.5% to 50% were selected .A control was also maintained simultaneously .There mosquito larva were not feed during experimental period .The number of mosquito larva dead or alive was noted & the pH, temperature was recorded.

LC50 values can be calculated by following method:

1) Graphical method:

Estimation of the LC50 involves plotting the data on semi logarithmic coordinate paper with concentration of chemical substance to Percentage of dead Mosquito larva. Thus, mortality observed in each tank plotted against the concentration in the same tank. A straight line is drawn between the two points representing the percentage dead at the two successive concentration that were lethal to more than half and to less than half of the test animals the concentration at which this line crosses the 50% lethality line is the essential 50value .

2) Statistical method:

Based on the data obtained from acute toxicity test, time dependent LC_{50} values and their 95% confidence limits can be calculated by any of a variety of statistical method but most widely used method are Probit analysis in Probit analysis method, the logarithmic values are read for each concentration/exposed and Probit values are read of the for the mortality. Then graph is plotted

RESULT:

***Ocimum sanctum*:**

Sr.No.	Extract of stock (ml)	Concentration of Extract in ppm	log of conc. of Extract	Percent Mortality	Probit value
1.	control	0	0.0	00	00
2.	0.5	2	0.3010	00	4.01
3.	1.0	4	0.6020	16.66	4.01
4.	2.0	8	0.9030	33.33	4.56
5	3.0	12	1.0791	66.66	5.71
6.	4.0	15	1.1139	83.33	5.95
7.	5.0	18	1.2552	99.9	8.09

Table: showing the LC_{50}*Ocimum sanctum* extract.

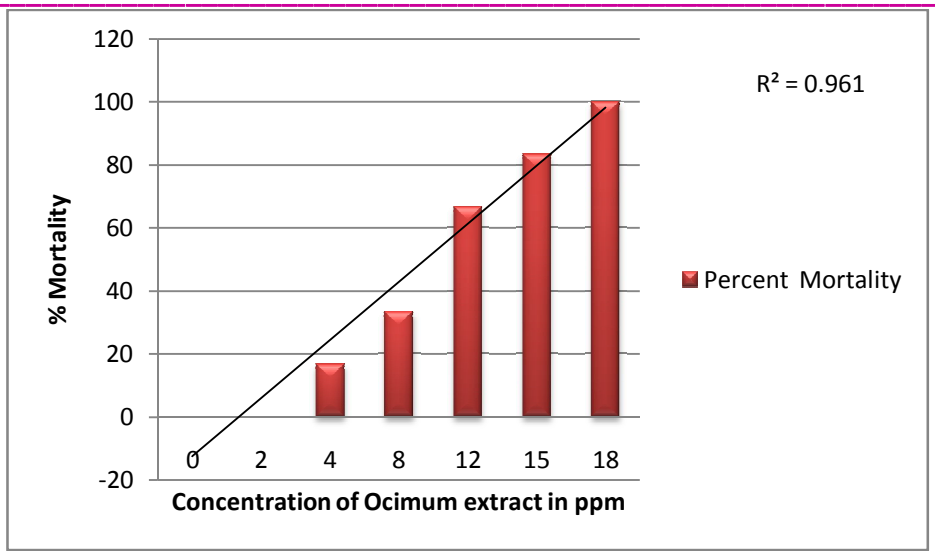


Fig. Histogram showing the effect of ***Ocimum sanctum*** on ***Aedes aegypti*** larva. As the concentration of increases, the percent mortality increases.

CONCLUSION:

By analyzing the results obtained from present study one can easily understand the effect of ***Ocimum sanctum*** on ***Aedes aegypti*** larvae. As the leaf extract of ***Ocimum sanctum*** are highly toxic at high doses these plants may eventually prove to be useful for Larvicidal activity. Optimum dosage, responsible for Larvicidal and adult emergence inhibition activity in ***Aedes aegypti.*** These plants would be eco-friendly and may serve as suitable alternative to synthetic insecticides as they are relatively safe and easily available.

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