

## Mosquito larvicidal activity of *Ocimum sanctum* on *Culex quinquefasciatus*

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### ABSTRACT

Use of chemical pesticides for a longer period posed number of health problems to the non-target organisms including human beings. Moreover, mosquitoes have developed resistance to the pesticides. Plant products are considered quite effective owing to their target species specific activity as well as their eco-friendly nature. In this context *Ocimum sanctum* of family-Lamiaceae have been tried for the phytoecdysis like compounds and their effect on II and IV instar larvae of *Culex quinquefasciatus*. It was noticed that IV instar larvae are more susceptible than II instar. *Ocimum sanctum* which is known as Tulsi in Hindi is found plentifully in local area. Dried leaves powder was Soxhleted for 48 hours duration in petroleum ether which yielded 2.76% green viscous crude substance. This yielded a crystalline substance  $\beta$ -ocimol - a phytoecdysone by spectral analysis. Its effect on larvicidal potential leading to delayed pupation and fall in population of the vector have been reported in the present paper. Therefore, it is suggested that plant products may be an alternative to the chemical pesticides provided they have this potentiality at low concentration. The LC<sub>50</sub> and LC<sub>90</sub> value for II and IV instar larvae of *Culex quinquefasciatus* recorded as 288.76 and 186.0 and 489.73 and 284.56 respectively.

**Key words :** *Ocimum*, *Culex*,  $\beta$ -ocimol, Ecdysis, Larvicidal.

### INTRODUCTION

Mosquito borne diseases such as malaria, filaria, dengue, yellow fever and recently outbreaken chikunguniya are still continuing to be a major health problem in tropical and subtropical countries<sup>1</sup>. Larval control during the past had been dependent mainly on the use of chemicals such as BHC and Paris green<sup>2</sup>. Use of these chemical pesticides for a longer period posed number of health problems to the non-target organisms including human being. Moreover, mosquitoes have developed resistance to the pesticides. Therefore, it is necessary that alternate larvicidal compounds be evaluated for future use. In the present laboratory for the two decades more than twelve plant crude extracts have been evaluated against II and IV instar larvae of *Culex quinquefasciatus* as well as *Anopheles stephensi*<sup>3-7</sup> which have shown antijuvenile activity, loss in fecundity and fertility including some developmental

defects. George and Vincent<sup>2</sup> have reported the comparative efficacy of three plant extracts against *Culex quinquefasciatus*. Similarly, Madhumathy<sup>8</sup> have evaluated the larvicidal potential of *Capsicum* against *Anopheles stephensi* and *Culex quinquefasciatus*.

Plant products are considered quite effective owing to their target species specific activity as well as their eco-friendly nature. In this context *Ocimum sanctum* of family Lamiaceae have been tried for the phytoecdysis like compounds and their effect on II and IV instar larvae of *Culex quinquefasciatus*.

### MATERIAL AND METHODS

#### Bioassay

Field collected *Culex quinquefasciatus* larvae were reared under laboratory conditions and

**Table 1 : Showing the effect of  $\beta$ -ocimol on growth, moulting and fall in population of II instar larvae of *Culex quinquefasciatus*.**

S. No.	Conc. in ppm.	24 hours larval mortality	Percentage of pupation	Percentage of adult emerged	Fall in population
1.	100	20%	40	24	76%
2.	200	38%	30	14	86%
3.	300	52%	28	12	88%
4.	500	96%	14	04	96%
5.	Control	04%	96	96	04%
6.	Untreated	1.0%	98	98	2%

**Table 2: Showing the effect of  $\beta$ -ocimol on growth, moulting and fall in population of IV instar larvae of *Culex quinquefasciatus*.**

S. No.	Conc. in ppm.	24 hours larval mortality	Percentage of pupation	Percentage of adult emerged	Fall in population
1.	100	34%	24	24	76%
2.	200	58%	20	14	86%
3.	300	96%	18	04	96%
4.	500	100%	Nil	Nil	100%
5.	Control	3.4%	96.6	96.6	3.4%
6.	Untreated	Nil	100	100	Nil

laboratory cultured stock were used for experimental bioassay. 25 second and early fourth instar larvae of *Culex quinquefasciatus* have been used in 500 ml. glass beaker to which 249 ml. of tap water and 1 ml. test concentration was added to each replicate in 100 to 500 ppm. concentration. Experiment was conducted in three replicates with one control and one untreated.  $LC_{50}$  value was calculated by using profit analysis method of Finney<sup>9</sup>. Larval bioassay was conducted according to the standard WHO method<sup>10</sup>.

#### Plant material

*Ocimum sanctum* commonly known as *Tulsi* in Hindi, is found plentifully in gardens. Plant samples were collected from botanical garden and identified by taxonomists. A voucher specimen was deposited in the herbarium record at serial No. 12.

#### Extraction and Isolation of phytoecdysone

700 gm. of dried leaves powder was Soxhleted for 48 hours duration in petroleum ether which yielded 2.76% green viscous crude substance. This was purified using TLC of silica gel which gave four compounds of which compound with pale yellow colour with RF value 0.57 was used for experimental bioassay. This yielded a crystalline substance  $\beta$ -ocimol - a phytoecdysone with melting point 280°C. 100 to 500 ppm. concentration was used for experimental bioassay.

#### RESULTS AND DISCUSSION

*Ocimum sanctum* which is a sacred plant, used traditionally for cough, cold and asthma since time immortal. It has been reported earlier<sup>11</sup> to possess a Ocimol compound causing ecdysis failure

in silk moth. Looking to this, its effect on larvicidal potential leading to delayed pupation and fall in population of the vector carrying filariasis have been reported in the present paper.

It was noticed that the b-ocimol isolated from *Ocimum sanctum* caused delayed pupation, pupal mortality, larval mortality as well as fall in population. LC<sub>50</sub> and LC<sub>90</sub> value when calculated by profit analysis were found to be 288.76 and 489.73ppm for II and 186.0 and 284.56 ppm for IV instar larvae respectively. It was noticed that majority of adult died during ecdysis. The larval-pupal intermedia, half ecdysed adults were noticed in the present study. Treated larvae experienced an increased larval period as compared to the control and untreated larvae. Due to death in the larval stage and moulting in to the pupa and adult, there was considerable fall in vector population with this compound. Similar results with *Annona squamosa* extract have been reported by Saxena et al<sup>7</sup>. George and Vincent<sup>2</sup> have reported the synergistic activity of three plant extracts, but they have mentioned that plant extracts at higher concentration makes them uneconomical for field use. Madhumathy<sup>8</sup> et. al have

also reported the larvicidal activity of ethanolic extract of *Capsicum annum* against IV instar larvae of *Culex quinquefasciatus* and *Anopheles stephensi* where they have noted more than 99% mortality at 0.024% concentration of *Capsicum annum* extract. It was also noticed in the present study that after the treatment of b-ocimol, the larvae become slowly inactive after 10 to 12 hours of the treatment. They perform Zig Zag motion instead coming up thoroughly. Therefore it can be suggested that plant products can be an alternative to the chemical pesticides provided they have this potentiality at low concentration. They are less toxic to the mammals, easily biodegradable in the ecosystem and quickly metabolized in the liver causing no ill effects. Hence can be recommended for field trials.

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#### REFERENCES

1. Das M.K., Ansari M.A. Evaluation of repellent action of *Cymbopogon martinii martinii* stapf var sofia oid against *Anopheles sundiacus* in tribal villages of Car Nicobar Island, Andman & Nicobar Island, India. *J. Vect. Borne Dis*; **40**(3-4): 101-105 (2003).
2. Susan George and S. Vincent. Comparative efficacy of *Annona squamosa* Linn. and *Pongamia glabra* vent. to *Azadirachta indica* A. Juss against mosquitoes. *J. vect. Borne Dis*; **42**: 159 - 163 (2005).
3. Saxena Rashmi, Saxena R.C. and Sharma C.L. *Biological control of mosquito vector. Him. J. Env. Zool.*, **9**: 47-48 (1995).
4. Saxena R.C., Harshan V., Saxena A., Sukumaran P., Sharma M.C. and Lakshamana Kumar M. Larvicidal and chemosterilant activity of *Annona squamosa* alkaloids against *Anopheles stephensi*. *J. of the American Mosquito Control Association*. **9**: 84-87 (1993).
5. Saxena R.C., Dixit O.P. and Padma Sukumaran. Laboratory assessment of indigenous plant extracts for anti-juvenile hormone activity on *Culex quinque fasciatus*. *Indian J. Med. Res.*, **95** : 204-206 (1992).
6. Anita Saxena and R.C. Saxena. Effects of *Ageratum conyzoides* extract on the development stages of malaria vector, *Anopheles stephensi* (Diptera : Culicidae). *J. Environ. Biol.*, **13**: 207-208 (1992).
7. Anita Saxena, V. Harshan and R.C. Saxena. Mosquito larvicidal activity of *Annona squamosa* extract. *German Journal for Applied Zoology.*, **79** : 185-192 (1993).
8. Madhumathy A.P., Ali Ashraf Alvazi and

- Vijayan V.A. Larvicidal efficacy of *Capsicum. annum* against *Anopheles stephensi* and *Culex quinquefasciatus*. *J. Vect. Borne Dis*; **44**: 223-226 (2007).
9. Finney D.J. Probit Analysis, III edn. London Cambridg University Press. 25-325 (1971).
10. Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/ VBC 81-807 (1981) .
11. Goyal S.K. To study on the nutritional management of *Morus alba* family Moraceae for the improvement of cocoon. *Ph. D. Thesis*, Barkatullah University, India. 139 (2005).