

**STUDIES ON ESSENTIAL OIL BASED FORMULATIONS
FOR CONTROLLING MOSQUITO LARVAE**

by

MOHINI TIWARY

Centre for Rural Development and Technology

Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

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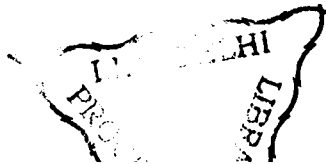
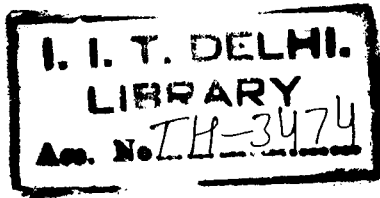


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CERTIFICATE

This is to certify that the thesis entitled “**Studies on essential oil based formulations for controlling mosquito larvae**” being submitted by **Mrs. Mohini Tiwary** to the Indian Institute of Technology, Delhi for the award of Doctor of Philosophy is a record of bonafide research work carried out by her under the guidance and supervision in conformity with the rules and regulations of Indian Institute of Technology, Delhi.

The research report and results presented in this thesis have not been submitted, in part or in full, to any other university or institute for the award of any degree or diploma.



(Dr. S.N. Naik)

Associate Professor
Centre for Rural Development and Technology
Indian Institute of Technology, Delhi
New Delhi- 110 016

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(Mohini Tewary)

ABSTRACT

Mosquitoes are well known for their public health importance because of their ability to act as vectors of pathogens causing malaria, dengue, yellow fever, encephalitis, filariasis and other related diseases, which affect many millions of people world over. The key efforts towards combating the mosquito borne diseases include disruption of their life cycle through the application of larvicidal agents with a preference over targeting the adult mosquitoes. Worldwide, larviciding relies primarily on use of chemical or biological insecticides. Though these larvicidal agents have had notable success, loss of efficacy because of resistance, stringent regulations on their use due to the associated health and ecological problems and/ or cost of some of these materials make them not only unsuitable for mosquito control but also limit the choice of mosquito larvicidal agents in future. In this scenario, there has been increasing interest in developing alternative larvicidal agents using locally available plant source, which may address some of the aforementioned problems associated with the use of chemical and biological larvicides and help reducing heavy reliance on them. Against this backdrop, studies were carried out on various aspects to explore the possibility of developing plant oil based larvicidal agents and assessing the practicality of their application in larval breeding sites.

Based on the medicinal and bioactive properties, out of the eight plant essential oils viz. Anethum sowa (seeds), Artemisia nilagirica (leaves), Cymbopogon citratus (leaves), Cyperus scariosus (tubers), Saussurea costus (roots), Trachyspermum ammi (fruits),

Valeriana jatamansi (rhizome) and *Zanthoxylum armatum* (fruits) selected for initial screening against the larvae of the three mosquito species (*Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*), essential oils from three plants viz. *Z. armatum*, *T. ammi* and *A. sowa* demonstrated very good larvicidal potential. *An. stephensi* showed greater tolerance towards all the essential oils tested. Larval sensitivity towards *T. ammi* and *A. sowa* were in the order *Ae. aegypti* > *Cx. quinquefasciatus* > *An. stephensi* whereas towards *Z. armatum* oil, *Cx. quinquefasciatus* was more sensitive followed by *Ae. aegypti*. The LC_{50} values of *Z. armatum*, *T. ammi* and *A. sowa* were in the range 49-58, 44-49 and 20-29ppm respectively. Thus, in general, the order of efficacy of the oils was *A. sowa* > *T. ammi* > *Z. armatum*.

GC/GC-MS study showed that essential oil from *Z. armatum* consisted mainly of oxygenated monoterpenes (68.4%) and monoterpenes (22.0%) with linalool (57.0%) and limonene (19.8%) as the major components respectively. *T. ammi* essential oil consisted mainly of monoterpene hydrocarbons (58.6%) and oxygenated monoterpenes (39.95%) with *P*-cymene (29.65%) and thymol (~40%) as the major components respectively. In *A. sowa* oil, dihydrocarvone (16%), carvone (27%) and dillapiol (~17%) were the major constituents.

Larvicidal activity was evaluated of the major compounds present in the essential oils viz. linalool (*Z. armatum*) thymol (*T. ammi*), and dillapiol (*A. sowa*). Linalool failed to produce mortality against any of the mosquito larvae to an appreciable degree. Thymol and dillapiol demonstrated promising larvicidal activity against all the mosquito species

with LC₅₀ values in the range 36-65 and 16-28ppm respectively. However, activity of the essential oils was not linearly dependent on these constituents.

In binary combination (LC₅₀ doses mixed 1+1) of essential oils, the mixture of A. sowa+T. ammi emerged as the most promising combination (mortality, 77-83%) against the mosquito larvae. Similarly, dillapiol invariably enhanced the efficacy of the pure compounds. LC₅₀ combination (1:1; v/v) of thymol+dillapiol was the most toxic (mortality, 83%) followed by carvacrol+dillapiol and limonene+dillapiol with a mortality of 71% and 67% respectively. Mixing dillapiol in the essential oils (Z. armatum and T. ammi) almost doubled their efficacy (mortality>94%) in the LC₅₀ concentration ratios of 3:1 to 4:1 against Ae. aegypti and Cx. quinquefasciatus respectively, thus allowing reduction of the amount nearly by half to achieve the satisfactory level of efficacy. The efficacy of the mixture of dillapiol and neem oil at a fixed test dose of 80ppm was comparable to that of the absolute dillapiol on replacing even one third of it by neem oil.

Liquid formulations (Emulsifiable Concentrate, EC and Microemulsion, ME) and granular formulation (Na-Alg beads) were developed for application of representative active ingredient, neem oil and dillapiol in the ratio of 3:1 (NOD) to evaluate the bioefficacy of such products under simulated field conditions against Laboratory Collected and Field Collected Larvae. Response of the liquid formulations (NOD EC 20 and NOD ME 5) was far superior (mortality range ~91 to 98%) to that of the encapsulated formulation (Na-Alg beads) with mortality in the range ~66 to 68% at the

end of nine days observations. Residual bioefficacy results indicated that up to the third day of incubation under simulated field conditions, bioefficacy of NOD ME 5 was maintained with total cumulative mortality of the larvae above 90%. Bioefficacy-shelf life study showed that storage period up to three months under ambient conditions did not affect the bioefficacy of NOD ME 5. Though formulation showed some toxicity against non-target biota, in view of the better efficacy, emulsion stability and environmental profile, NOD ME holds considerable promise for its practical utility in controlling mosquito larvae particularly in small breeding places that appear seasonally for short time and therefore treatment area is limited and manageable.

Overall, the present study brings to the fore the mosquito larvicidal potential of essential oils and their compounds and underlines the strategy to make the product viable through integrating other abundantly available bioactive plant oils. Analysis, based on demands for natural products and prevailing costs showed that such products may be produced at village level involving the activities viz. cultivation or collection of the plant materials, establishment of units for making quality intermediates and formulations which ensure the scope of establishing viable enterprise and generating income and employment opportunities in rural areas. Thus a sustainable system may be evolved using plant-based resources for tackling mosquito borne problems.

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