



## STUDIES ON LARVICIDAL ACTIVITY OF PLANT ESSENTIAL OIL FORMULATION AGAINST THE EGGS OF IMPORTANT VECTOR MOSQUITOES, *ANOPHELES STEPHENSI* (LISTON), *CULEX QUINQUEFASCIATUS* (SAY) AND *Aedes Aegypti* (L.) AT LABORATORY CONDITION

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### ABSTRACT

In the present investigation Plant Oil Formulation (POF) with different concentrations were tested for its larvicidal activity against the eggs of *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say) and *Aedes aegypti* (L.). Results clearly indicated that among the five concentrations tested (15.62, 31.25, 62.5, 125 and 250 ppm) maximum cumulative mortality of eggs were obtained at 250 ppm concentration of POF against the fourth instar larvae of the selected species. The present investigation paves the way for further exploration of possible way of utilizing the POF in IPM to control the important vector mosquitoes.

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**Key words:** *Embllica officinalis* (EOL), mild steel, adsorption isotherm, corrosion inhibition.

### INTRODUCTION

Mosquitoes are important vectors of several tropical diseases, including malaria, filariasis, and numerous viral diseases, such as dengue, Japanese encephalitis and yellow fever. In countries with a temperate climate they are more important as nuisance pests than as vectors. (Jaswanth *et al.*, 2002). There are about 3000 species of mosquito, of which about 100 are vectors of human diseases. Control measures are generally directed against only one or a few of the most important species and can be aimed at the adults or the larvae. (Kambooj, 2000). Mosquitoes are also becoming increasingly resistant to traditional chemical pesticides and there is growing concern about the potential health and environmental risks surrounding these products. Environmental protection agencies have banned or placed severe restrictions on the use of many pesticides which were formerly used in mosquito control programmes and there are now fewer adulticides available than there have been for the last 20 years (Carvalho, *et al.*, 2008). It is likely, therefore, that mosquitoes will very quickly develop high levels of resistance to the remaining available adulticides, leading to concern among operational mosquito control personnel that effective insecticides may not be available in the near future (Kline, 1994). Hence, it is imperative that novel mosquito control methods are developed and put into general use as soon as possible.

One potential alternative approach to the use of chemical pesticides is the use plant secondary metabolites like active compounds and other volatile oils (Rihana, 1993). Recently natural products of plants are widely under investigation against insects due to their excellent properties like cheap availability and renewable nature, presence of an array of characters like insecticidal, antifeedant, ovicidal etc., and their environmental safety nature (Saxena and Thikku, 1988 and 1990). Earlier works of several authors revealed that botanicals can have strong larvicidal (Mwangi and Rembold (1988); Vasudevan *et al.* (1989); Mohsen *et al.*, (1990); Insun *et al.* (1999); Anyanwu *et al.* (2001) Carvalho *et al.* (2003)) oviposition deterrent and ovicidal activity (Millar *et al.* (1992); Su and Mulla (1998 & 1999); Ritchie (2001); Poonam *et al.* (2002)). In the present study essential oils of six plants commonly found in Tamilnadu, India were tested against third instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*.

### 2. MATERIALS AND METHODS

Larvicidal bioassay - Larvae of *Ae. aegypti*, *An. stephensi* and *C. quinquefasciatus* were collected from stock culture. The Plant Oil Formulation (POF) was tested for its larvicidal activity against 25 numbers of late third instar larvae of *An. stephensi*, *C. quinquefasciatus* and *Ae. aegypti* by the standard procedure of WHO (1996). The Plant Oil Formulation was volumetrically diluted to 500 mL with dechlorinated water to obtain the test solution of

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250, 125, 62.5, 31.25 and 15.25 ppm and Tween20 served as a control. The experiments were carried out  $28 \pm 2$  °C. For each dose four replicates were maintained. The larval mortality data were recorded after 24 h of treatment using the formula of Abbot (1925). The LC<sub>50</sub> was carried out by Probit Analysis (Finney, 1971) and the level of significance was found out by Duncan's Multiple Range Test (Duncan, 1963).

### 3. RESULTS AND DISCUSSION

The consequences of different concentrations of the POF viz. 15.62, 31.25, 62.5, 125.0 and 250.0 ppm on the larvicidal activity against *An. stephensi* were depicted in Table 2 and the data revealed that the highest larval mortality of 99.2 % was observed at 250 ppm concentration, whereas the lowest mortality of 24.16 % was noted at 15.62 ppm concentration. The mortality of 52.66, 68.0 and 84.5 % were observed at 31.25, 62.5 and 125.0 ppm concentration respectively. In the control, the total mortality of 1.16 % was observed. The 24 h LC<sub>50</sub> and LC<sub>90</sub> values of the POF were 35.95 and 138.86 ppm respectively. The Chi-square value was 1.735 and it indicated that the larvicidal activity was significant at 0.05 % level.

**Table 1** List of plant volatile oils used in the preliminary screening against three different species of mosquitoes.

| Common Name    | Botanical Name               | Larvicidal activity (%) of 1000 ppm at 24 hr. |
|----------------|------------------------------|---|
| Calamus oil    | <i>Acorus calamus</i>        | 100   |
| Camphor oil    | <i>Cinamomum camphora</i>    | 60  |
| Cinamon oil    | <i>Cinamomum veerun</i>      | 100   |
| Clove oil      | <i>Myrtus caryophyllus</i>   | 100   |
| Eucalyptus oil | <i>Eucalyptus globulus</i>   | 100   |
| Lemon oil      | <i>Citrus limon (medica)</i> | 100   |

**Table 2** Larvicidal activity of plant oil formulation against fourth instar larvae of *Anopheles stephensi*

| Concentration (ppm) | Mortality (%) | 95% Confidence limit |       |       |                     |        | LC <sub>90</sub> (ppm) | Chi-square X <sup>2</sup> |
|---------------------|---------------|----------------------|-------|-------|---------------------|--------|------------------------|---------------------------|
|                     |               | LC50 (ppm)           | Lower | Upper | Regression equation |        |                        |                           |
| 15.62               | 24.16 ± 2.01  |                      |       |       |                     |        |                        |                           |
| 31.25               | 52.66 ± 6.10  |                      |       |       |                     |        |                        |                           |
| 62.5                | 68.0 ± 4.88   |                      |       |       |                     |        |                        |                           |
| 125                 | 84.5 ± 5.17   | 35.95                | 23.82 | 48.93 | Y=1.857+2.065X      | 138.86 | 1.735                  |                           |
| 250                 | 99.2 ± 9.66   |                      |       |       |                     |        |                        |                           |
| Control             | 1.16 ± 0.09   |                      |       |       |                     |        |                        |                           |

Values represent mean ± S. D.

**Table 3** Larvicidal activity of plant oil formulation against fourth instar larvae of *Culex quinquefasciatus*

| Concentration (ppm) | Mortality (%) | LC50 (ppm) | Lower | Upper | Regression equation | LC90 (ppm) | Chi-square X <sup>2</sup> |
|---------------------|---------------|------------|-------|-------|---------------------|------------|---------------------------|
| 15.62               | 20.16 ± 1.14  |            |       |       |                     |            |                           |
| 31.25               | 48.66 ± 1.19  |            |       |       |                     |            |                           |
| 62.5                | 64.0 ± 2.47   |            |       |       |                     |            |                           |
| 125                 | 76.5 ± 5.07   | 42.17      | 28.12 | 57.77 | Y=1.848+1.966X      | 170.25     | 3.235                     |
| 250                 | 99.0 ± 3.27   |            |       |       |                     |            |                           |
| Control             | 1.16 ± 0.08   |            |       |       |                     |            |                           |

Values represent mean ± S. D.

The effect of different concentration of the POF against *C. quinquefasciatus* was presented in Table 3. The percentage of larval mortality was found to be maximum of 99.0 % at 250 ppm concentration of the plant oil formulation. The total mortality of 1.16 % was recorded in Tween 20, which served as a control. The LC<sub>50</sub> and LC<sub>90</sub> values of Plant oil formulation 42.17 and 170.25 ppm respectively. The 95 % of LCL and UCL were 28.12 and 57.77 ppm respectively. The Chi-square value was 3.23, which indicated that the larvicidal activity was significant at 0.05 % level.

The efficacy of different concentrations of the POF viz. 15.62, 31.25, 62.5, 125.0 and 250.0 ppm on the larvicidal activity against *Ae. aegypti* was furnished in Table 4. The larval mortality of 48.66, 64.0 and 80.5 % were observed at 31.25, 62.5 and 125 ppm concentration respectively. The LC<sub>50</sub> and LC<sub>90</sub> values of the POF 42.17 and 170.25 ppm respectively. The 95 % confidence limit of LCL and UCL were 28.120 and 57.774 ppm respectively. The Chi-square value was 3.235 which indicate significant larvicidal activity at 0.05 % level. These findings are in agree with the earlier findings of Carvalho *et al* (2003), who have been reported that the essential oils obtained from *Lippia sidoides* controlled *Ae. aegypti* larvae significantly. Similar results were also obtained by various earlier workers (Ciccia *et al.*, 2000; Ezeonu *et al.*, 2001).

The results showed that the mortality of the larvae increased as the doses of the sample were increased. The same trend was observed among the three mosquito species. Further, it was observed that many larvae were failed to ecdyze to perfect pupae producing larval-pupal intermediate (Mwangi and Mukiyama, 1988). These results are very promising in creating new effective and affordable approaches to the control of vector mosquitoes.

**Table 4** Larvicidal activity of plant oil formulation against fourth instar larvae of *Aedes aegypti*

| Concentration (ppm) | Mortality (%) | LC <sub>50</sub> (ppm) | Lower | Upper | Regression equation | LC <sub>90</sub> (ppm) | Chi-square X <sup>2</sup> |
|---------------------|---------------|------------------------|-------|-------|---------------------|------------------------|---------------------------|
| 15.62               | 20.16 ± 1.24  |                        |       |       |                     |                        |                           |
| 31.25               | 48.66 ± 2.10  |                        |       |       |                     |                        |                           |
| 62.5                | 64.0 ± 1.19   | 42.17                  | 28.12 | 57.77 | Y=1.7+2.08X         | 170.25                 | 3.235                     |
| 125                 | 80.5 ± 4.13   |                        |       |       |                     |                        |                           |
| 250                 | 99.2 ± 5.04   |                        |       |       |                     |                        |                           |
| Control             | 1.16 ± 0.08   |                        |       |       |                     |                        |                           |

Values represent mean ± S. D.

It is interesting to note that, the following observations were made among the experimental larvae i.e., the sluggish movement and peculiar coiling of treated larvae seem to suggest some neutral or muscular disturbance by some active principle; which might be cause acute lethal effect these findings is in corroborate with the observation of Kiamuddein *et al* (1979). The detail lethal effect of compound is more likely to be caused by a disturbance of the endocrine mechanism that regulate moulting and metamorphosis. This mechanism of action has been postulated previously for Neem Seed Kernel Extract (NSKE) by Zebitz (1986).

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