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RESEARCH ARTICLE

# STUDIES ON OVICIDAL 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 ovicidal 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 eggs 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.



## INTRODUCTION

Mosquitoes are still a major problem of the world, particularly tropical and subtropical regions. Because mosquitoes are responsible for the transmission of various dreadful deseases (WHO. 1996). Many vectors and pest species have developed physiological resistance to several chemical compounds applied to control those organisms (Brown, 1986). Furthermore, potent and also more expensive chemical compounds that replace the less toxic ones have also failed due to resistant gained by vectors. Despite the plant world comprises a rich storehouse of bio-chemicals that could be tapped and used as pesticides. The toxic constituents present in the plants represent the secondary metabolites and have only an insignificant role in primary physiological process that synthesizes them (Venkatachalam, 2001). Recently natural products of plants are widely under investigation against insect 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;1990 and Pierce *et al.,* 1991). Hence, this study is an attempt to check the bioefficacy of some plant oils (Volatile oils) formulations for their ovicidal activity against the freshly laid eggs of vector mosquitoes *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say) and *Aedes aegypti* (L.).

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## MATERIALS AND METHODS

Egg rafts were drawn from mosquito colonies of *A. stephensi, C. quinquefasciatus* and *A. aegypti.* Freshly laid eggs of 0 to 18 h old were collected separately by providing ovitraps in mosquito cages from 16: 00 10: 00 h overnight. Ovitraps were kept in the cages after 2 days of blood given female mosquitoes. The different concentrations viz. 250, 125, 12.5, 6.25 and 3.125 ppm of the plant oil formulation were used in this study. Stock solution of the oil formulation was prepared with methanol. In the case of *A. aegypti* and *A. stephensi,* the eggs were laid on filter paper lining provided in the ovitraps. These filter paper strips containing eggs after of *C. quinquefasciatus* were carefully removed from the piece of filter paper with a brush. The eggs of the age group 0 to 18 h were exposed at different concentrations of the plant oil formulation separately. In each treatment, minimum of 100 eggs was used. A parallel control experiment was setup and each experiment was replicated six times. After the exposure period, the eggs were thoroughly rinsed with water and left in enamel bowls containing water for hatchiag. Parallel control experiments were performed using the same number of eggs in untreated water. The number of eggs hatched was scored and the percentage hatchability was calculated using the control data. In order to study the delayed effects of the treatment of egg stage, the larvae hatched out from the treated eggs were separately reared till the adult stage. Mortality with larval, pupal and adult stages were calculated and corrected with control by Abbott's (1925) formula.

## RESULT AND DISCUSSION

Initially preliminary study was conducted on selected individual oils at 1000ppm concentration for their ovicidal activity and the results were given in Table 1. The treatment of eggs of *A. stephensi* with different concentrations of the plant oil formulation caused embryonic death resulting in the failure of hatching. In the present study, different concentrations used were 15.62, 31.25. 62.5, 125 and 250 ppm. The 0 -18h old eggs were selected and treated for 6 h and 18 h. In the 6 h treatment of 0 – 18 h old eggs, the cumulative mortality of 61.0, 72.0, 86.0, 97.1 and 100 % was observed at 15.62, 31. 25, 62.5 125 and 250 ppm concentrations respectively and in the control only 6.4 % cumulative mortality were noticed. In the case of 18 h treatment, the cumulative mortality of 66.2, 74.4, 95.0, 100 and 100 % was observed at 15.62, 31.25, 62.5, 125 and 250 ppm concentrations respectively, with a control of 8.1 % cumulative mortality (Table 1 and 2).

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

| Common Name | Botanical Name | Ovicidal activity (%) of 1000 ppm at 24 hr. |
|---|---|---|
| Calamus oil | *Acorus calamus* | 100 |
| Camphor oil | *Cinamomum camphora* | 80 |
| Cinamon oil | *Cinamomum veerum* | 100 |
| Clove oil | *Myrtus caryophyllus* | 100 |
| Eucalyptus oil | *Eucalyptus globulus* | 100 |
| Lemon oil | *Citrus limon (medica)* | 100 |

However, the larvae, which hatched out from the treated eggs showed higher mortality in all the treatments. Our findings are in parallel with the earlier studies of Bassole *et al*. (2003) who have reported that the essential volatile oils of *Cymbopogon* showed significant ovicidal activity against the eggs of *A. gambiae* and *A. aegypti.*

The treatment of *C. quinquefasciatus* eggs with various concentrations of the plant oil formulation caused embryonic death resulting in the failure to hatch and the cumulative mortality of 59.7, 67.9, 83.6 and 95.0 % was noticed at 15.62, 31.25, 62.5 and 125 ppm concentrations respectively, whereas the cumulative mortality of 7.8 % was observed in the control. In the case of 18 h treatment 100% mortality was observed at 15.62, 31.25, 62.5 and 12.5 ppm concentrations respectively whereas the control showed only 8.0 % cumulative mortality (Table 3). Earlier similar results have been obtained by Su and Mulla (1998) against *Culex* sp by azadirachtin. In general, 250 ppm concentration of the plant oil formulation was more toxic compared to other concentrations. Egg mortality in any of the treatments did not go beyond 50.0 % even with the higher concentration of 250 ppm. However, the larvae, which hatched out from the treated eggs, showed much higher levels of mortality in all the treatments. Also, the treatments produced low to moderate levels of pupal mortality or adult mortality at the time of adult emergence.

**Table 2. Effect of the plant oil formulation on ovicidal activity of 0 - 18 hours old eggs of *Anopheles stephensi* treated for 6 and 18 hours treatment**

| Concentration (ppm) | Treated for 6 h | | | | Treated for 18 h | | | |
|---|---|---|---|---|---|---|---|---|
| | Egg Mortality (%) | Larval Mortality (%) | Pupal/ Adult Mortality (%) | Cumulative Mortality (%) | Egg Mortality (%) | Larval Mortality (%) | Pupal/ Adult Mortality (%) | Cumulative Mortality (%) |
| 15.62 | 11.8 ± 0.08 | 24.2 ± 1.47 | 25.0 ± 2.03 | 61.0 | 13.2 ± 2.11 | 24.4 ± 2.21 | 28.6 ± 0.08 | 66.2 |
| 31.25 | 15.6 ± 1.99 | 27.4 ± 1.08 | 29.0 ± 1.47 | 72.0 | 17.5 ± 2.03 | 30.8 ± 2.24 | 26.4 ± 1.07 | 74.4 |
| 62.5 | 27..2 ± 1..23 | 36.4 ± 2.54 | 22.5 ± 1.87 | 86.0 | 32.6 ± 1.77 | 44.4 ± 3.11 | 18.0 ± 2.04 | 95.0 |
| 125 | 33.4 ± 1.19 | 54.2 ± 6.04 | 9.5 ± 0.64 | 97.1 | 36.0 ± 1.05 | 54.0 ± 4.01 | 10.0 ± 0.06 | 100 |
| 250 | 47.0 ± 0.77 | 51.0 ± 2.21 | 2.0 ± 0.08 | 100 | 49.0 ± 1.99 | 51.0 ± 1.09 | 0.00 | 100 |
| Control | 1.4 ± 0 .06 | 1.2 ± .009 | 3.8 ± 0.002 | 6.4 | 1.5 ± 2.01 | 3.0 ± 0.04 | 3.6 ± 0.04 | 8.1 |

Values represent mean ± S. D.

**Table 3. Effect of the plant oil formulation on ovicidal activity of 0 - 18 hours old eggs of *Culex quinquefasciatus* treated for 6 and 18 hours treatment**

| Concentration (ppm) | Treated for 6 h | | | | Treated for 18 h | | | |
|---|---|---|---|---|---|---|---|---|
| | Egg Mortality %) | Larval Mortality (%) | Pupal/Adult Mortality (%) | Cumulative Mortality (%) | Egg Mortality (%) | Larval Mortality (%) | Pupal/Adult Mortality (%) | Cumulative Mortality (%) |
| 15.62 | 11.2 ±1.44 | 23.5 ± 2.11 | 25.0 ± 2.27 | 59.7 | 15.0 ± 1.08 | 23.7 ± 1.76 | 25.3 ± 1.07 | 64.0 |
| 31.25 | 15.4 ± 1.87 | 25.0 ± 1.11 | 27.5 ± 1.16 | 67.9 | 17.6 ± 1.03 | 28.4 ± 1.19 | 30.0 ± 2.24 | 76 .0 |
| 62.5 | 21.3 ± 0.99 | 36.5 ± 1.09 | 25.8 ± 1.73 | 83.6 | 26.3 ± 1.17 | 34.7 ± 1.07 | 31.2 ± 3.14 | 92.2 |
| 125 | 28.2 ± 1.04 | 58.8 ± 4.03 | 8.0 ± 0.09 | 95.0 | 32.0 ± 2.07 | 52.2 ± 2.01 | 10.0 ± 1.14 | 100 |
| 250 | 42.7 ± 2.21 | 56.3 ± 1.19 | 0.00 | 100 | 43.5 ± 2.44 | 56.5 ± 5.03 | 0.00 | 100 |
| Control | 1.2 ± 0.07 | 2.2 ±0.77 | 4.4 ±0.98 | 7.8 | 2.6 ± 0.17 | 3.2 ± 0.08 | 2.2 ± 2.27 | 8.0 |

Values represent mean ± S. D.

**Table 4. Effect of the plant oil formulation on ovicidal activity of 0 - 18 hours old eggs of *Aedes aegypti* treated for 6 and 18 hours treatment**

| Concentration (ppm) | Treated for 6 h | | | | Treated for 18 h | | | |
|---|---|---|---|---|---|---|---|---|
| | Egg Mortality (%) | Larval Mortality (%) | Pupal/Adult Mortality (%) | Cumulative Mortality (%) | Egg Mortality (%) | Larval Mortality (%) | Pupal/Adult Mortality (%) | Cumulative Mortality (%) |
| 15.62 | 11.5 ± 1.05 | 23 ± 1.42 | 25.0 ± 0.07 | 59.5 | 15.2 ± 0.08 | 24.5 ± 1.00 | 25.1 ± 1.16 | 64.8 ± 7.49 |
| 31.25 | 16.2 ± 0.99 | 25.4 ± 2.23 | 26.5 ± 1.05 | 68.1 | 17.6 ± 1.07 | 27.2 ± 1.65 | 31.3 ± 1.21 | 75.8 ± 9.14 |
| 62.5 | 21.5 ± 1.07 | 36.5 ± 2.78 | 24.5 ± 1.11 | 82.5 | 27.8 ± 2.04 | 34.5 ± 2.11 | 33.5 ± 3.07 | 95.8 ± 9.77 |
| 125 | 31.4 ± 1.16 | 56 ± 1.47 | 9.6 ± 0.09 | 97.0 | 33.4 ± 1.17 | 41.2 ± 2.34 | 21.0 ± 2.07 | 96.2 ± 5.47 |
| 250 | 44.5 ± 2.02 | 55.5 ± 5.01 | 0.0 ± 0.00 | 100 | 44.6 ± 1.19 | 54.4 ± 4.08 | 0 ± 0.00 | 100 ± 0.00 |
| Control | 1.2 ± 0.05 | 2 ± 0.05 | 3.3 ± 0.08 | 6.5 | 2.0 ± 0.05 | 3.2 ± 0.03 | 2.0 ± 0.06 | 7.2 ± 0.07 |

Values represent mean ± S. D.

The eggs of *Aedes* treated with different concentrations of the plant oil formulation caused embryonic death resulting in failure to hatch the eggs. The 0 -18 h old eggs treated with different concentrations of the Plant oil formulation for 6 h exhibited 100 % cumulative mortality at 250 ppm concentration. The cumulative mortality of 59.5, 68.1, 82.5 and 97.0 % was observed at 15.62, 31.25, 62.5 and 125 ppm concentration respectively, with 6.5 % cumulative mortality in the control. In the case of 18 h treatment 100 % mortality was observed at 125 and 250 ppm concentration the cumulative 12.5 ppm concentration respectively whereas the control revealed 8.4 % cumulative mortality (Table 4). The treatment had deleterious effects causing high larval mortality and moderate pupal or adult mortality in the larvae hatched out of the treated eggs. The mortality depends on the relationship between the age of egg rafts, concentration of the plant oil formulation an effects causing high larval mortality and moderate pupal or adult mortality in the larvae hatched out of the treated eggs. The mortality depends on the relationship between the age of egg rafts, concentration of the plant oil formulation and the period of exposure.

As a consequence of factors such as, strict environmental legislation, increased resistance of pest to synthetic pesticids, growing residue awareness among consumers, mounting industrial research and development cost of chemical insecticides, there has been shift towards the interest for the use of natural insecticides (Van Latum and Gerritis, 2007). The results of the present study are interesting. It is evident from the present data that the exposure of mosquito eggs to the plant oil formulation elicits not only egg mortality but also delayed effects resulting in mortality at larval, pupal and adult stages. Though the percentage of ovicidal activity is moderate, an important finding is that the larvae which hatched out of the treated eggs immediately succumbed to death. Exposure of freshly laid eggs to the plant oil formulation was more effective that the older eggs. Miura *et al*. (1976) showed that the age of embryos at the time of treatment played a crucial role with regard to the effectiveness of the chitin synthesis inhibitor, dimilin to *C. quinquefasciatus*. Exposure time also has a vital role in causing toxicity. According to smith and salkeld (1966), differences in susceptibility to ovicides are due to differential rates of uptake, penetration through the chrocion, conversion to active inhibitor; detoxication and failure of the toxic into reach the target.

Elumalai *et al* (2004) observed that the efficacy to act on the embryo inside the egg shell depends on an efficient penetration of the insecticide, which in turn is influenced by the exposure period which they have observed it against the armyworm, *Spodoptera litura,* the same trend was observed in the present study also against the selected species of vector mosquitoes. The results clearly disclosed that the treatment of 6h was found to be less effective in inducing higher rates of mortality as compared to 18h treatment. Ovicidal action of Anopheles stephensi at 6h exposure period showed the cumulative mortality of 97.1 % at 125 ppm but in 18 h exposure period the cumulative mortality was 100 %. In the case of *Culex quinquefasciatus* and Aedes aegypti, 125 ppm concentrat on at 6 h exposure period showed the cumulative mortality as 95.0 and 97.0 % respectively. At 18 h treatment the cumulative mortality was 100 and 96.2 % respectively. The same trend was noticed in the egg mortality also.

In this study, the concentration of essential oil also played a crucial role with regard to the effectiveness of ovicidal activity. The highest cumulative mortality was observed in the highest concentration of the plant oil formulation. Broadbent and Prec (1984) reported more entry of the chemical inside the eggshell, when eggs were directly exposed to higher concentrations of the compounds, which affected the embryogenesis. Similarly longer exposure periods also facilitated the increased penetration of the compounds into the shell, thus increasing their effectiveness. Therefore the current study clearly indicated that the ovicidal activity of the plant oil formulation against the egg rafts of *An. stephensi*, *C. quinquefasciatus* and *Aedes* depends upon three key factors viz., concentrations of the plant oil formulation, age of egg rafts and period of exposure. The eggs of mosquitoes are found to be much tolerant to the action of insecticides compared to the larval stages.

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