

# Larvicidal Activity Of Plant Oil Formulation Against Three Important Vector Mosquito Sp.

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## Citation

A Elangovan, G Veeraiyan, K Elumalai, M Prakash. *Larvicidal Activity Of Plant Oil Formulation Against Three Important Vector Mosquito Sp.*. The Internet Journal of Veterinary Medicine. 2008 Volume 6 Number 1.

## Abstract

Over a last few decades, there has been increasing interest in isolating biological substances against various diseases. Of which mosquitoes plays a vital role in transmitting numerous diseases that are harmful to human being (malaria, filariasis and numerous viral diseases, such as dengue, Japanese encephalitis and yellow fever). Mosquitoes are also become increasingly resistant to traditional chemical pesticides and there is growing concern about the potential health and environmental risks surrounding these products. In order to overcome this, efforts have been taken to control the mosquito by using biological substances. In the present study plant oil formulation was used to control the mosquito larvae. The efficacy of different concentration of the plant oil formulation viz. 15.62, 31.25, 62.5, 125.0 and 250.0 ppm for their larvicidal activity against *Ae. aegypti*, *An. stephensi* and *C. quinquefasciatus*. These results are very promising in creating new effective and affordable approaches to the control of vector mosquitoes.

## 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 (Poonam et al., 2002). 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 fourth instar larvae of, *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*.

## 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 fourth 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 250, 125, 62.5, 31.25 and 15.62 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  $LC_{50}$  was carried out by Probit Analysis (Finney, 1971) and the level of significance was found out by Duncan's Multiple Range Test (Duncan, 1963).

## 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  $LC_{50}$  and  $LC_{90}$  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.

### Figure 1

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 verum</i>	100
Clove oil	<i>Myrtus caryophyllus</i>	100
Eucalyptus oil	<i>Eucalyptus globulus</i>	100
Lemon oil	<i>Citrus limon (medica)</i>	10

### Figure 2

Table 2: Larvicidal activity of plant oil formulation against fourth instar larvae of

Concentration (ppm)	Mortality (%)	95% Confidence limit				
		$LC_{50}$ (ppm)	Lower	Upper	Regression equation	Chi-square (X <sup>2</sup> )value
15.62	24.16 $\pm$ 2.01	35.95	23.82	48.93	$Y=1.857+2.065X$	1.735
31.25	52.66 $\pm$ 6.10					
62.5	68.0 $\pm$ 4.88					
125	84.5 $\pm$ 5.17					
250	99.2 $\pm$ 9.66					
Control	1.16 $\pm$ 0.09					

Values represent mean  $\pm$  S. D.

### Figure 3

Table 3: Larvicidal activity of plant oil formulation against fourth instar larvae of

Concentration (ppm)	Mortality (%)	$LC_{50}$ (ppm)	Lower	Upper	Regression equation	$LC_{90}$ (ppm)	Chi-square (X <sup>2</sup> )value
15.62	20.16 $\pm$ 1.14	42.17	28.12	57.77	$Y=1.848+1.966X$	170.25	3.235
31.25	48.66 $\pm$ 1.19						
62.5	64.0 $\pm$ 2.47						
125	76.5 $\pm$ 5.07						
250	99.0 $\pm$ 3.27						
Control	1.16 $\pm$ 0.08						

Values represent mean  $\pm$  S. D.

### Figure 4

Table 4: Larvicidal activity of plant oil formulation against fourth instar larvae of

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

Values represent mean  $\pm$  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  $LC_{50}$  and  $LC_{90}$  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  $LC_{50}$  and  $LC_{90}$  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 (2008), 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. 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 Mwangi and Rembold (1988). 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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