

LARVICIDAL EFFICACY OF PLANT OILS AGAINST THE MALARIAL VECTOR, ANOPHELES STEPHENSI (DIPTERA: CULICIDAE)

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Article Received on
15 March 2018,

Revised on 05 April 2018,
Accepted on 25 April 2018,

DOI: 10.20959/wjpr20189-12162

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ABSTRACT

Malaria is a dreadful disease caused by female vector mosquito, *Anopheles stephensi*. Control of *An. stephensi* by various synthetic chemicals produced instant results, however, they cause severe and deleterious effects on human health too. Thus, in the present study, an attempt was made to study the mosquito larvicidal activity of selected plant oils against the 4th instar larvae of *An. stephensi*. The experimental data revealed that there was a significant larvicidal activity induced by the plant oils among the experimental larvae.

KEYWORDS: Plant oil, *Anopheles stephensi*, larvicidal activity, botanical control.

INTRODUCTION

Mosquitoes are insects belonging to the order Diptera and as other true flies; develop through four distinct life stages-egg, larva, pupa and adult. It is hard to comprehend the amount of disease and the resulting sickness, death and economic loss caused by the mosquito.^[1] Their attacks on farm animals can cause loss of weight and decreased milk production. Some mosquitoes are capable of transmitting diseases such as malaria, yellow fever and dengue. Malaria is transmitted by different *Anopheles* species, depending on the region and the environment.^[2]

To control mosquito, any type of control should involve careful consideration of the biology of the mosquitoes and be based on scientific surveillance. A response to control nuisance mosquitoes may look very different from a response to control disease-vectoring mosquitoes.^[3] In all cases, larval mosquito control should be considered as the first option for

abatement. This involves location of larval habitats, followed by their modification or treatment in such a way that the integrity of the habitat is preserved but the mosquitolarvae are reduced in numbers.^[4] By controlling larval mosquitoes, the adults may never become a problem. Larviciding has the greatest control impact on mosquito populations because the larvae are concentrated, immobile and accessible. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water and thus, it is easy to deal with them in this habitat.

The use of conventional pesticides in the water sources, however, introduces many risks to people and/or the environment. Aromatic plants and their essential oils are very important sources of many compounds that are used in different respects. Essential oils and plant extracts are still an important natural resource and more promising for pesticides or insecticides.^[5] My present investigation demonstrates that the efficacy of effective nine essential oils in killing the larval *An. Stephensi*, under laboratory conditions.

MATERIALS AND METHODS

Essential oils

Essential oils were obtained from Tegraj & Co (P) Ltd, India (commercial producers of plant essential oils and aromatic substances) were used in this study.

Test organism

The larval stages of *An. stephensi* culture was raised at $28\pm 2^{\circ}\text{C}$, 65-70% RH with a photo period of $11\pm 0.5\text{h}$. The reared larvae were fed on dog biscuits and yeast powder in the ratio of 3:1 in plastic tray (24x35x5cm). Adults were fed with 10% sucrose solution. In addition to sucrose feeding, the female mosquitoes were also fed with live rat blood every four days as to produces satisfactory number of eggs and then larvae for the testing. The fourth instar larvae were used for the bioassays.

Bioassay Procedure

Larvicidal activity was determined by following the standard procedure (WHO, 1982). Effective oils were tested at five different concentrations viz, 100,200,300,400 and 500 ppm. Twenty numbers of fourth instar larvae of *An. stephensi* were kept in a 500 ml glass beaker containing 249 ml of dechlorinated water and 1.0 ml of desired plant essential oil concentrations. Four replicates for each concentration were maintained. In control 1.0 ml of Tween80 (0.01%) was added. Mortality of larvae was recorded after 24 hrs of treatment.

While recording the percentage mortalities for each concentration, the moribund and dead larvae in five replicates were combined. It has been described that dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region; moribund larvae are those incapable of rising to the surface. The lethal concentration (LC50) and LC90 values and 95% of lower and upper confidence limits were calculated by using probit analysis (Finney, 1971). Mortality was corrected by using Abbott formula (Abbott, 1925).

$$\text{Abbotts Percent Corrected Mortality} = \frac{\% \text{ MT} - \% \text{ MC}}{100 - \% \text{ MC}} \times 100$$

Where,

% CM = % Corrected Mortality;

% MT = % Mortality in treated;

% MC = % Mortality in control.

Statistical analysis

Calculation of LC50 and statistical analysis LC50 values (the concentration at which 50% of the larvae were mortality) were calculated by probit analysis using software Statistical tools.

RESULT AND DISCUSSION

The percent mortality values of 4th instar larvae treated with different concentration (ranging from 100 to 500 ppm) of the ten essential oils after 24 hrs treatment are represented Table 1 for *An.Stephensi*. The LC50 and LC90 values between the concentration of essential oils and 24 hrs percent mortality of 4th instar larvae of *An.Stephensi* are represented in Table 2 respectively. Table 1 show the larvicidal activity of effective ten essential oils against the fourth instar *An.Stephensi* larvae. All the ten oils showed concentration dependent larval mortality. Catnip oil was the most effective treatment. It shows maximum activity against fourth instar larvae of *An.Stephensi*. The minimum Larvicidal activity was recorded in cornmint oil.

Table 2 show the lethal concentration of effective oils against *An.Stephensi* larvae. The lowest LC50 of 352.60 ppm was recorded in catnip oil. The LC90 for catnip oil was recorded as 580.51ppm. The highest LC50 and LC90 values of 526.80 and 789.29 ppm were recorded in cornmint oil. Our data clearly illustrate that selected effective oils were more potent larvicidal on *An.Stephensi*. Our data also show a dose-dependent effect on mortality, with

increasing concentrations of essential oil and its essential oils increasing mortality of the larvae.

The findings of the present investigation indicate that larvicidal properties of ten essential oils on *An.Stephensi* reported in the present study, confirm their potential for control of the mosquito populations. These properties can be exploited to protect the environment of the user or the user from vectors of disease.

Previous studies regarding the extracts and essential oils of *Melissa*, *Rosemary*, *Lavender*, *lime* and *ginger* that have been done by others support our study and they have also proposed the essential oils as a suitable substitution of chemical repellent (Oshaghi, 2003; Rajkumar, 2007; Barat, 2012; Kweka, 2009). In the animal experiments, *Lavender* and *Eucalyptus* oils rather than other oils had a better repellent effectiveness, 97.16 and 97.15% respectively, against *anopheles* (Gillij, 2007). Therefore, they could be recommended as a safe and suitable substitution of chemical repellent. In this work, we have tested the repellents against only one species and do not know if these compounds are protective against other mosquito species or medically important insects. By more clinical trial we may introduce the essential oils in the insect's repellent herbal cream formulation. Different factors may interfere in insect's repellent efficacy that the main factor could be effective substances of essential oils and extracts. Therefore, analysis of different fractions of herbal oils and extracts and its effect on the insects is recommended.

Most of these essential oils are highly volatile and this contributes to their poor longevity as mosquito repellents. However, this problem can be addressed by using fixatives or careful formulation to improve their longevity. For example, oils from turmeric and hairy basil with addition of 5% vanillin repelled 3 species of mosquitoes under cage conditions for a period of 6 to 8 h depending on the mosquito species (Tawatsin, 2001). The exception to this is para-menthane 3, 8 diol which has a lower vapour pressure than volatile monoterpenes found in most plant oils (Barasa, 2002) and provides very high protection from a broad range of insect vectors over several hours (Carroll, 2006).

The plants can be used alone or combined for effective protection against mosquitoes. They can also be used for control of mosquito breeding (Barnard, 2004; Trongtokit, 2005). They also offer safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost. However, toxicity tests of the active plants need to be

done to ascertain their safety in administration (Robert, 1991; Rutledge, 1978).

Table 1: Larvicidal efficacy of plant oils against *An. stephensi* larvae.

Plant oil	Scientific name	Concentration (ppm) Mean larval mortality \pm S.D.				
		100	200	300	400	500
Catnip	N. cataria	7.5 \pm 0.5	10.6 \pm 0.8	18.6 \pm 0.5	20.8 \pm 1.5	24.2 \pm 0.9
Thyme	T. vulgaris	7.5 \pm 0.577	11.5 \pm 1.290	17.5 \pm 1.290	20.5 \pm 0.577	24 \pm 0.816
Basil	O.basilicum	7 \pm 0.816	11 \pm 0.816	17.75 \pm 0.5	20.5 \pm 0.577	23.5 \pm 0.577
Patchouli	P. cablin	7.25 \pm 0.5	11.5 \pm 1.290	16.75 \pm 0.957	18.75 \pm 0.5	22 \pm 0.816
Rosemary	R. officinalis	7.25 \pm 0.5	11.5 \pm 1.290	14.25 \pm 0.957	18.25 \pm 0.957	21.75 \pm 0.957
Spearmint	M. spicata	7.25 \pm 0.5	11.5 \pm 1.290	16.75 \pm 0.957	18.75 \pm 0.5	22 \pm 0.816
Lavender	L.angustifolia	6.25 \pm 0.5	9.75 \pm 0.5	11.75 \pm 0.5	14.25 \pm 0.957	17.75 \pm 0.957
Marjoram	T.mastichina	6.75 \pm 0.957	10.25 \pm 0.5	12.75 \pm 1.258	15 \pm 0.816	17.25 \pm 0.957
Clary sage	S.sclarea	7 \pm 1.414	11 \pm 0.816	14.75 \pm 1.25	17.5 \pm 1.290	22 \pm 1.414
Cornmint	M. arvensis	5 \pm 1.414	9.5 \pm 0.816	10.75 \pm 1.25	15.5 \pm 1.290	20 \pm 1.414
Controle		0.0 \pm 0.0				

Table 2: Probit analysis of plant oils tested on *Aedes aegypti* fourth instar larvae.

Plant oil	Scientific name	LC ₅₀	LC ₉₀
Catnip	N. cataria	352.60	580.51
Thyme	T. vulgaris	370.53	616.40
Basil	O.basilicum	395.12	640.34
Patchouli	P. cablin	416.99	661.98
Rosemary	R. officinalis	438.19	680.37
Spearmint	M. spicata	445.77	700.81
Lavender	L.angustifolia	467.98	731.57
Marjoram	T.mastichina	492.66	757.02
Clary sage	S.sclarea	512.14	773.12
Cornmint	M. arvensis	526.80	789.29

ACKNOWLEDGEMENT

Authors are very much grateful to UGC, New Delhi for the financial assistance and also thankful to the Principal, and Head, Department of Zoology, Government Arts College for men (Autonomous), Nandanam, Chennai-35 for providing the laboratory facilities.

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