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LARVICIDAL ACTIVITY OF ESSENTIAL OILS AGAINST *Aedes aegypti* and *Culex quinquefasciatus* LARVAE (DIPTERA: CULICIDAE)

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ABSTRACT

Five essential oils from various parts of five plant species i.e. *Acorus calamus ,Mentha arvensis, Ocimum basilicum, Saussrea lappa* and *Cymbopogan citrates* were investigated for their larvicidal property against *Aedes aegypti* (L.) and *Culex quinquefaciatus* (Say) larvae. Essential oils were obtained by steam distillation method. The mosquitoes were reared in laboratory by maintaining conditions and twenty late 3rd instar larvae of *Ae. aegypti* (L.) and *Cx. quinquefaciatus* (Say) were exposed to different concentrations of essential oils ranging from 1.95-1000.00ppm. The larval mortality was observed after 24 hours under the laboratory conditions. Results showed that all the tested essential oils produced significant larval mortality against two mosquito species. However, the highest larvicidal activity was observed in the essential oil from *O. basilicum* against *Ae. Aegypti* (L.) and *Cx. quinquefaciatus* (Say) with LC_{50} values 75.35 ppm and 92.30 ppm respectively. However the LC_{50} values for *A. calamus, M. arvensis, S. lappa and C. citrates* against *Ae. Aegypti* (L.) were 99.41, 114.33,128.89 and 136.28 ppm respectively and against *Cx. quinquefaciatus* (Say) were 107.81, 112.18, 141.43 and 148.54ppm respectively. No mortality was observed in controls. According to the larvicidal activity of essential oils against *Ae. aegypti* (L.) and *Cx. quinquefaciatus* (Say), the oils were arranged in the following ascending order of preference i.e. *O. basilicum> A. calamus> M. arvensis> S. lappa> C. citrates*. From the results, it can be concluded that essential oils had excellent potential for controlling mosquito larvae in Pakistan.

Key words: essential oil, *Aedes aegypti, Culex quinquefasciatus.*

INTRODUCTION

The most important single group of insects in terms of Public health importance are mosquitoes. Blood feeding female mosquitoes are responsible for the transmission of a large number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of deaths every year (Rahuman *et al*., 2008). *Aedes aegypti* (L), the yellow fever mosquito is the vector of dengue. It is widely distributed in the tropical and subtropical zones (Hales *et al*., 2002). *Ae. aegypti* (L.) is very closely associated with the human habitat. The geographical range of *Ae .aegypti* (L.) is increasing in part due to rapid urbanization and increased global movement of people and cargo (Kyle and Harris, 2008). *Cx. quinquefasciatus* (Say), the potential vector of lymphatic filariasis, is the most widely distributed tropical disease with around 120 million people infected worldwide and 44 million people having common chronic manifestation (Bernhard *et al*., 2003).

The eradication of these diseases largely relied on interruption of disease transmission cycle. It can be controlled by either targeting the mosquito larvae through spraying of stagnant water breeding sites or by killing the adult mosquitoes using insecticides (Joseph *et al*., 2004). Larviciding is a successful way of reducing mosquito population in their breeding places before they emerge into adults. The prevention of mosquito breeding through the use of larvicides is the most effective way to fight with this mosquito importunation. Synthetic insecticides have been used as larvicide in several countries for the last 30 years (Chavasse and Yap, 1997). However, the non-selectiveness of insecticides and harmful effects on other organisms is the major hindrance with the use of these chemical insecticides (De Omena *et al*., 2007). The need for development of effective insecticides should be taken into consideration due to the toxicity problems, together with the increased incidence of insect resistance. In most parts of the world, Synthetic chemical larvicides continue to be applied for controlling mosquitoes but many of these chemicals are toxic to human, animal and plant life and resistance can be problematic in regulating the control. Therefore, researchers are currently exploiting natural substances to be used as insecticides for controlling larval mosquitoes. (Moretti *et al*., 2002; Cetin *et al*., 2004). In Pakistan studies have been conducted on biopotential of *Ocimum sanctum* as toxicant and repellent against termite, *Heterotermes indicola* (Wasmann) (Isoptera: Rhinotermitidae) (Manzoor *et al.*, 2011)

In view of recently increased interest in developing plant origin insecticides as an alternative to chemical insecticides, this study was undertaken to access the toxicant potential of the five essential oils from *A.*

calamus, M. arvensis, O. basilicum, S. lappa and *C. citrates* against mosquito species *Ae. aegypti* (L.) and *Cx. quinquefasciatus* (Say). The results of present study showed an ecofriendly approach for larvicidal activity of essential oils against mosquito larvae.

MATERIALS AND METHODS

Plant materials: Following plants / plant parts were collected from their natural habitats: roots of *Acorus calamus* (Sweet flag) (Fam.: Araceae) from Peshawar, roots of *Saussurea lappa* (Costus) (Asteraceae) from Kashmir leaves of *Mentha arvensis* (Mint) (Lamiaceae), leaves of *Ocimum basilicum* (Sweet basil) (Lamiaceae), and leaves of *Cymbopogan citrates* (Lemon grass) (Apocynaceae) from Lahore. All plants were authenticated by a plant taxonomist from the Department of Botany, Lahore College For Women University, Lahore.

Extraction: The leaves and roots were washed with tap water, shade-dried, and finely grounded. The finely ground plant leaf powder was placed in a round bottom flask and water was added. The Dean- Stark trap was connected to flask and a water condensor was attached with trap for condensing the vapours. The flask was heated and the distillate collected in the trap automatically recirculated into distillation flask and oil started collecting in it. Heating was continued until no further increase in amount of oil observed. The essential oils were separated from water, dried over anhydrous sodium sulphate and stored at 4°C for further experimentation.

Test organisms: The mosquitoes of *Ae. aegypti* (L.) (Yellow fever mosquito) and *Cx. quinquefasciatus* (Say) (Brown house mosquito) were reared in Entomology Research Laboratory, Department of Zoology, Lahore College for Women University. The male mosquitoes were fed on 6% glucose solution soaked in cotton pad while the females were fed on blood diet twice a week by hand feeding or by rat feeding. Larvae were fed daily with finely ground mixture containing equal parts by weight of wheat germ, beef liver and yeast. Mosquitoes were held at 28-30 °C, 75 ± 5% relative humidity (RH), with a photo period of 14-h light, 10-h dark.

Preparation of the oil solution: A sufficient amount of target oil was dissolved in distilled water using 2 ml of 100% acetone to produce a stock solution at 1000ppm. This solution was used to prepare the serial dilutions of target oil at concentration of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.90, 0.97ppm through dilution of the stock solution with distilled water; three replicate of each concentration were made. In addition to three replicates, the control contains 0 ppm of oil, 2ml of 100% acetone and distilled water (Xue *et al*., 2001).

Bioassay Larvicidal: Each replicate containing 200 ml of the described oil solution was placed in a 250-ml plastic cup. Twenty late 3rd instars larvae of target mosquitoes were transferred into each cup (Mohtar *et al*., 1999). Three replicates were set up for each concentration, and an equal number of controls were set up simultaneously using tap water. After that, the beakers were left on the laboratory table for 24 h. The number of dead larvae in each beaker was as counted after 24 hours.

Calculation of LC_{50} and statistical analysis: Insect mortality data were corrected by Abbott's formula (1925), LC_{50} values (the concentration at which 50% of the larvae were immobilized) were calculated by probit analysis using the PROBIT software Statistical Package. Analysis of Variance was performed by Probit analysis. Means were compared with Duncan's Multiple Range test. 95% of upper confidence limit (UCL), lower confidence limit (LCL) and Chi-square were also calculated. Results with $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

As discussed in materials and methods, the results of relative toxicity of five essential oils against *Ae. aegypti* (L.) and *Cx. quinquefasciatus* (Say) after 24 hours of treatment are presented in Table 1. It was evident from table that all the tested essential oils demonstrated significant larvicidal activity against *Ae. aegypti* (L.) and *Cx. quinquefasciatus* (Say). Among five essential oils the maximum efficacy was observed in the *O .basilicum* oil against *Ae. aegypti* (L.) and *Cx. quinquefasciatus* (Say) mosquito species with the LC_{50} values as 75.35 ppm and 92.30 ppm respectively. The Chi-square values are significant at $p< 0.0001$ level. The exposure time is very important in determining the LC_{50} values in tested oils. However, the LC_{50} values for *A. calamus, M. arvensis, S. lappa and C. citrates* against *Ae. aegypti* (L.) are 99.41, 114.33,128.89 and 136.28ppm respectively and against *Cx. quinquefasciatus* (Say) are 107.81, 112.18, 141.43 and 148.54ppm respectively. The Chi-square values in the bioassays indicated probably the heterogeneity of the test population. The 95% confidence limits (LC_{50} (LCL-UCL) were also calculated and values are shown in table 1.

Mean percentage mortality of five plant essential oils against *Ae. aegypti* (L.) and *Cx. quinquefasciatus* are presented in table 2 & 3 and are also compared with distilled water (control). No mortality was recorded in control treatments. As indicated there were no significant differences among essential oils from different plants for two mosquito species *Ae. aegypti* (L.) and *Cx. quinquefasciatus* (Say) in terms of larvicidal activity (Table 2 and 3). As expected, the percent larval mortality increased with increasing concentrations of the oil. At high concentration (1000ppm), mortality was not

significantly different. A concentration of 1000ppm of the oil was found to be 100% larvicidal. However, at a concentration of 250ppm the oil killed more than 50% of third late instars.

Table 1: Relative toxicity of five essential oils against *Ae.aegypti* (L.) and *Cx. quinquefasciatus* (Say) after 24 hours of treatment

Essential oils	Mosquito species	LC_{50} (ppm)	95% Confidence limits (ppm)	Fit of Probit line			
			LCL – UCL	Slope ± SE	X^2	df	p
A. calamus	*Ae. aegypti* (L.)	99.41	71.06 – 141.42	1.60±0.19	5.13	9, 20	P<0.0001
	Cx. quinquefasciatus (Say)	107.81	74.39 – 161.43	1.36±0.16	5.77	9, 20	P<0.0001
M. arvensis	*Ae. aegypti* (L.)	114.33	80.44 – 166.96	1.49±0.18	4.58	9, 20	P<0.0001
	Cx. quinquefasciatus (Say)	112.18	80.10 – 160.35	1.60 ±0.19	4.48	9, 20	P<0.0001
O. basilicum	*Ae. aegypti* (L.)	75.35	53.21 – 108.08	1.50±0.17	6.54	9, 20	P<0.0001
	Cx. quinquefasciatus (Say)	92.30	64.86 – 134.03	1.47±0.17	4.54	9, 20	P<0.0001
S. lappa	*Ae. aegypti* (L.)	128.89	95.12 – 176.72	1.95±0.24	2.28	9, 20	P<0.0001
	Cx. quinquefasciatus (Say)	141.43	99.56 – 208.10	1.51±0.18	4.77	9, 20	P<0.0001
C. citrates	*Ae. aegypti* (L.)	136.28	95.66 – 201.07	1.48 ±0.18	6.02	9, 20	P<0.0001
	Cx. quinquefasciatus (Say)	148.54	106.09 – 214.35	1.62±0.20	5.43	9, 20	P<0.0001

Table 2: Mean mortality ± SE of five plant essential oils at ten concentrations against *Ae. aegypti* (L.) when compared with distilled water (control).

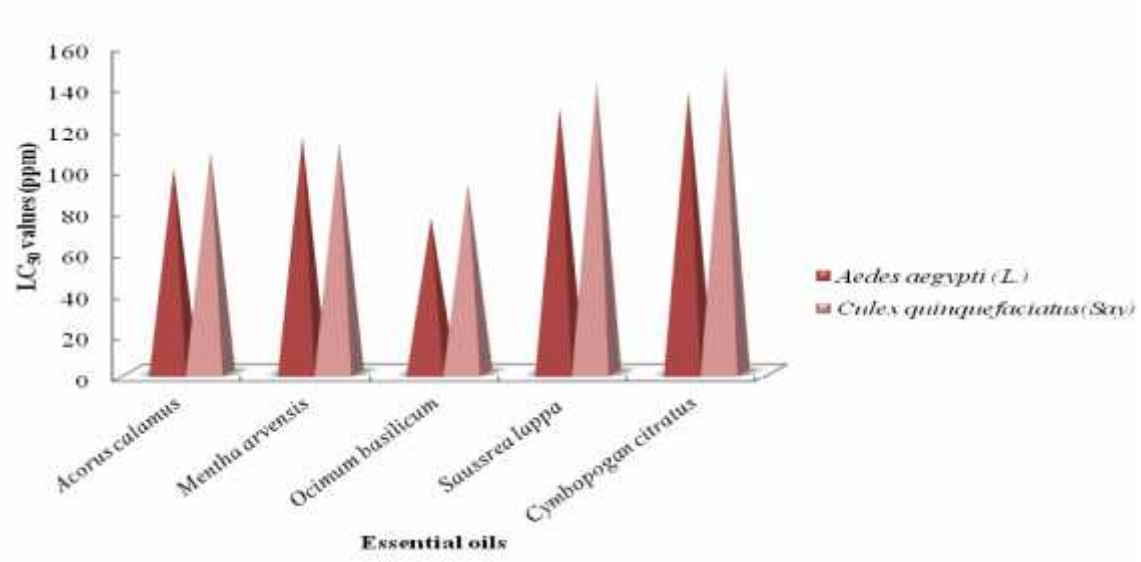
Concentrations (ppm)	*A. calamus*	*M. arvensis*	*O. basilicum*	*S. lappa*	*C. citrates*
1.95	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$
3.90	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$5.0\pm0.0^{b,B}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$
7.81	$0.0\pm0.0^{a,A}$	$5.0\pm0.0^{b,B}$	$10.0\pm0.0^{c,C}$	$0.0\pm0.0^{a,A}$	$5.0\pm0.0^{b,B}$
15.62	$15.0\pm0.5^{b,B}$	$15.0\pm0.5^{c,B}$	$20.0\pm0.5^{d,C}$	$5.0\pm0.0^{b,A}$	$15.0\pm0.0^{c,B}$
31.25	$30.0\pm0.0^{c,D}$	$25.0\pm0.5^{d,C}$	$30.0\pm0.0^{e,D}$	$15.0\pm0.0^{c,A}$	$20.0\pm0.5^{d,B}$
62.50	$40.0\pm0.5^{d,D}$	$35.0\pm0.5^{e,C}$	$35.0\pm0.0^{f,C}$	$30.0\pm0.5^{d,B}$	$25.0\pm0.0^{e,A}$
125.00	$50.0\pm0.5^{e,D}$	$40.0\pm0.0^{f,B}$	$45.0\pm0.0^{g,C}$	$40.0\pm0.0^{e,B}$	$35.0\pm0.5^{f,A}$
250.00	$65.0\pm0.5^{f,A}$	$65.0\pm0.5^{g,A}$	80.0 ± 0.0^{h}	70.0 ± 0.5^{f}	$65.0\pm0.5^{g,A}$
500.00	$85.0\pm0.5^{g,C}$	$80.0\pm0.0^{h,B}$	$95.0\pm0.5^{i,D}$	$85.0\pm0.5^{g,C}$	$75.0\pm0.5^{h,A}$
1000.00	$100.0\pm0.0^{h,A}$	$100.0\pm0.0^{i,A}$	$100.0\pm0.0^{j,A}$	$100.0\pm0.0^{h,A}$	$100.0\pm0.0^{i,A}$
Control	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$

Means followed by the same lower case letter within a given essential oil (columns) are significantly different (Duncan's multiple range test, $p< 0.05$); means followed by the same upper case letter at a given concentration (row) are significantly different (Duncan's multiple range test, $p< 0.05$).

Table 3: Mean mortality ± SE of five plant essential oils at ten concentrations against *Cx. quinquefasciatusis* (Say) when compared with distilled water control.

Concentrations (ppm)	*A. calamus*	*M. arvensis*	*O. basilicum*	*S. lappa*	*C. citrates*
1.95	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$
3.90	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$
7.81	$10.0\pm0.0^{b,B}$	$5.0\pm0.0^{b,A}$	$10.0\pm0.0^{b,B}$	$5.0\pm0.0^{b,A}$	$5.0\pm0.0^{b,A}$
15.62	$20.0\pm0.5^{c,C}$	$15.0\pm0.0^{c,B}$	$15.0\pm0.0^{c,B}$	$10.0\pm0.0^{c,A}$	$10.0\pm0.0^{c,A}$
31.25	$25.0\pm0.0^{d,C}$	$20.0\pm0.5^{d,B}$	$30.0\pm0.5^{d,D}$	$20.0\pm0.5^{d,B}$	$15.0\pm0.0^{d,A}$
62.50	$35.0\pm0.5^{e,C}$	$30.0\pm0.0^{e,B}$	$40.0\pm0.0^{e,D}$	$30.0\pm0.0^{e,B}$	$20.0\pm0.5^{e,A}$
125.00	$45.0\pm0.5^{f,C}$	$40.0\pm0.5^{f,B}$	$45.0\pm0.0^{f,C}$	$35.0\pm0.5^{f,A}$	$35.0\pm0.5^{f,A}$
250.00	$60.0\pm0.0^{g,A}$	$70.0\pm0.5^{g,B}$	$70.0\pm0.5^{g,B}$	$60.0\pm0.5^{g,A}$	$60.0\pm0.5^{g,A}$
500.00	$80.0\pm0.0^{h,B}$	$85.0\pm0.5^{h,C}$	$85.0\pm0.5^{h,C}$	$75.0\pm0.5^{h,A}$	$80.0\pm0.5^{h,B}$
1000.00	$100.0\pm0.0^{i,A}$	$100.0\pm0.0^{i,A}$	$100.0\pm0.0^{i,A}$	$100.0\pm0.0^{i,A}$	$100.0\pm0.0^{i,A}$
Control	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$	$0.0\pm0.0^{a,A}$

Means followed by the same lower case letter within a given essential oil (columns) are significantly different (Duncan's multiple range test, $p< 0.05$); means followed by the same upper case letter at a given concentration (row) are significantly different (Duncan's multiple range test, $p< 0.05$).



When two species were compared for their toxicity for five essential oils. there were no significant ($p < 0.05$) differences in toxicity for all the tested oils ($p = 0.17$), but when the oils were compared for their toxicity, there were significant ($p < 0.05$) difference in the toxicity for all the tested essential oils ($p = 0.01$). Govindarajan, (2011) studied the larvicidal and repellent properties of essential oils from various parts of four plant species *C. citrates, Cinnamomum zeylanicum, Rosmarinus officinalis* and *Zingibe rofficinale against Culex tritaeniorhynchus* (*Cx. Tritaeniorhynchus*) and *Anopheles subpictus* (*An. subpictus*).Results showed that all the four plant essential oil produced significant larval mortality against two species. The LC_{50}value of *C. citrates* for *Culex tritaeniorhynchus* and *Anopheles subpictus* was 136.58 and 77.24ppm respectively. He concluded that these four essential oils including *C. citrates* had promising larvicidal repellent properties against *An. Subpictus* and *Cx. tritaeniorhynchus.*

The findings of our studies are also in conformity with Bhatnagar *et al.* (1993). They evaluated the insecticidal properties of essential oils and major constituents of aromatic plants, *O. basilicum* (L.) and *O. sanctum* (L.) against *An. stephensi* Liston, *Ae. aegypti* (L.) and *Cx. quinquefasciatus* (Say) mosquito species under laboratory conditions. The bioassay tests revealed that the essential oil of *O. basilicum* and its major constituent, methyl chavicol are more effective as compared to *O. sanctum.* Dosages of 0.003 ml/43.0 cm^2 of essential oil and 0.001 ml/43.0 cm^2 of methyl chavicol extracted from *O. basilicum* induced 100 per cent mortality in all the three mosquito species. So the essential oils and their major constituents of *O. basilicum* were more toxic to *An. stephensi*, followed by *Ae. Aegypti* (L.) and *Cx. quinquefasciatus* (Say) mosquitoes. They have reported *O. basilicum* oil as a promising mosquito larvicidal. So the present study has shown that five essential oils which were distilled from *A. calamus* , *M. arvensis, O. basilicum, S. lappa* and *C. citrates* has larvicidal activity against *Ae. aegypti* (L.) and *Cx. quinquefasciatus* (Say). It could be used selectively in places where water is stagnant and would affect the fertility of adults emerged from larvae exposed to oil. Further studies are needed on formulations against mosquitoes and their efficacy and cost effectiveness, the products based on these essential oils may contribute greatly to reduction in environmental chemicalisation and to an overall reduction of the population density of significant vectors of Dengue fever such as *Ae. aegypti* (L.).

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