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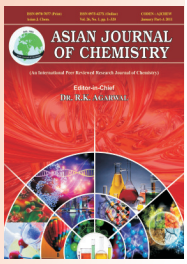

Evaluation of Essential Oil from Common Medicinal Plants Against *Culex quinquefasciatus* Larvae (Diptera: Culicidae) in Pakistan

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Five essential oils from various parts of plant species *E. citriodora*, *P. harmala*, *A. indica*, *C. nucifera* and *Turpentine* were investigated for their larvicidal properties against *Culex quinquefasciatus* (Say). Twenty late 3rd instar larvae of *Cx. quinquefasciatus* were collected from the insectaria and exposed to different concentrations of essential oils ranging from 1.95-1000 ppm. The larval mortality was observed after 24 h under the laboratory conditions. Results showed that the highest larvicidal activity was in *Turpentine* oil against *Cx. quinquefasciatus* (Say) with the LC_{50} values 63.8 ppm. However the LC_{50} values for *E. citriodora*, *P. harmala*, *A. indica*, *C. nucifera* were 165.51, 85.91, 207.56 and 338.58 ppm, respectively. It was concluded that five essential oils which were distilled from *E. citriodora*, *P. harmala*, *A. indica*, *C. nucifera* and *Turpentine* had remarkable larvicidal properties, which may be considered as a potent source for the production of natural larvicides which would be environmentally safe and alternative to synthetic insecticides.

Keywords: Essential oil, Medicinal plants, *Culex quinquefasciatus* Larvae.

INTRODUCTION

A major cause of illness and death throughout the world are the diseases which are transmitted by insects[1]. According to public health the most important groups of insects are mosquitoes. They transmit a number of diseases such as filariasis, malaria, Japanese encephalitis, dengue, *etc*. over two billion people are in danger because of mosquito born diseases especially in tropical countries[2]. It is a strong winged domestic species seen all over the world and around human dwellings. Adult *Cx. quinquefasciatus* (Say) is approximately 3.96 to 4.25 mm in length[3]. The species is highly anthropophlic (they prefer human blood). The females will take multiple blood meals while male survive only on sugar meals. The prevention of mosquito breeding through the use of synthetic larvicides is the most effective way to get rid off these mosquitoes[4]. The 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 stage of mosquitoes[5,6].

Biopesticides are excellent substitute to synthetic pesticides because of their minor toxicity to humans, insignificant environmental pollution and other benefits[7]. Present study was carried out with the objective to evaluate the comparative efficacy of some essential oils against Lymphatic filariasis causing mosquito *Cx. quinquefasciatus*.

EXPERIMENTAL

All tests were conducted by using larvae of mosquito, *Cx. quinquefasciatus* were taken from insectaria. The identification was based on following morphological characters.

To evaluate the larvicidal activity of essential oils following plants were used *viz.*, *Eucalyptus citriodora* (Eucalyptus), *Peganum harmala* (Harmal), *Azadirachta indica* (Neem), *Cocos nucifera* (Coconut), *Turpentine* (Tarpeen).

Extraction of essential oils: Oils were extracted by steam distillation (Reverse Dean-Stark method).

Evaluation of essential oils with respect to their larvicidal properties: The test was conducted in Entomology Research laboratory, Zoology department, Lahore College for Women University at room temperature. The experimental plant essential oils were used in trials. Three replicates of each oil were prepared by dissolving the suitable amount of oil in distilled water to produce stock solution by using the acetone. Only 2 mL acetone and 198 mL of distilled water were used in the control replicates[8]. The stock solution of 1000 ppm was prepared by dissolving 1 mL of essential oil in 1000 mL of distilled water using 2 mL of 100 % acetone. This solution was used to prepare the serial dilutions of target oil in concentration of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.90,

0.97 ppm through dilution of the stock solution with distilled water; three replicates of each concentration were made. In addition to three replicates, the control contains 0 ppm of oil, 2 mL of 100 % acetone and distilled water.

Bioassay of oil solution: Each replicate containing 200 mL of the described oil solution was placed in a 250 mL of beakers. Twenty late 3rd instars larvae of culex mosquitoes were transferred into each beaker[9]. After that, the beakers were left on the laboratory table for 24 h. The number of dead larvae in each beaker was counted after 24 h. Twenty 3rd instar larvae of respective specie were transferred to each beaker. The number of dead larvae in each beaker was counted after 24 h of contact at room temperature. The larvae were considered dead if they were immobile and unable to reach the water surface.

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 PROBIT software Statistical Package for the Social Sciences while randomized complete blocked design ANOVA were used to detect the significant differences between the treatments in all tests. Means were compared with Duncan's multiple range tests.

RESULTS AND DISCUSSION

Public awareness and resulting environmental agencies ruling led to the removal of some synthetic insecticides from the market[10]. There are number of alternatives to using chemical pesticides for mosquito larval control. One of the alternative methods is the use of essential oils. In the view of recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to access the toxicant potential of the five essential oils that are *Turpentine*, *P. harmala*, *A. indica*, *E. citriodora* and *C. nucifera* against mosquito species *Cx. quinquefasciatus* (Say).

The percentage mortality of mosquito species *Cx. quinquefasciatus* when exposed to different concentrations of five essential oils *i.e.*, *E. citriodora*, *P. harmala*, *A. indica*, *C. nucifera* and *Turpentine* after 24 h exposure are shown in Table-1. The LC_{50} values and relative toxicity of these five essential oils are given in Table-2.

***Turpentine* oil treatment:** Table-1 revealed the percentage mortality of larvae of *Cx. quinquefasciatus* (Say) when exposed to different concentrations of *Turpentine* oil. The % mortality for *Cx. quinquefasciatus* (Say) after 24 h of treatment was 0, 0, 10, 30, 30, 45, 55, 75, 100 and 100 % at concentrations of 1.95, 3.90, 7.81, 15,62, 31.25, 62.50, 125.00, 250.00, 500.00 and 1000.00 ppm, respectively. The LC_{50} value (Table-2) for *Cx. quinquefasciatus* (Say) was 63.8 with lower and upper 95 % confidence limits as 402.530 and 1558.19, respectively. Analysis of variance revealed that concentrations of *Turpentine* oil tested against *Cx. quinquefasciatus* (Say) were significantly different among all treatments ($F = 3.233$, $df = 6,3$; $p < 0.0001$).

***Peganum harmala* oil treatment:** Table-1 revealed the percentage mortality of larvae of *Cx. quinquefasciatus* (Say) when exposed to different concentrations of *Peganumharmala* oil. The % mortality for *Cx. quinquefasciatus* (Say) after 24 h of treatment was 0, 0, 10, 10, 25, 45, 55, 75, 85 and 100 % at concentrations of 1.95, 3.90, 7.81, 15,62, 31.25, 62.50, 125.00, 250.00, 500.00 and 1000.00 ppm, respectively. Table-2 showed the LC_{50} value for *Cx. quinquefasciatus* (Say) was 85.91 with lower and upper 95 % confidence limits as 533.45 and 2171.539, respectively. Analysis of variance revealed that concentrations of *P. harmala* oil tested against *Cx. quinquefasciatus* (Say) were significantly different among all treatments ($F = 8237$, $df = 7,2$; $p < 0.0001$).

***Cocos nucifera* oil treatment:** Table-1 revealed the percentage mortality of larvae of *Cx. quenquefasciatus* (Say) when exposed to different concentrations of *C. nucifera* oil. The % mortality for *Cx. quinquefasciatus* (Say) after 24 h of treatment

TABLE-1
PERCENTAGE MORTALITY ± SE OF *Culex quinquefasciatus* WHEN EXPOSED TO DIFFERENT CONCONTRATIONS OF FIVE ESSENTIAL OILS AND CONTROL UNIT

S. No	Concentration (ppm)	% Mortality ± SE					
		Turpentine oil	*P. harmala* oil	*Cocos nucifera* oil	*Azadiracta indica* oil	*Eucalyptus citriodora* oil	Control
1	1.95	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	3.90	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3	7.81	10.0 ± 0.5	10.0 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4	15.62	30.0 ± 0.5	10.0 ± 0.5	0.0 ± 0.0	5.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5	31.25	30.0 ± 0.0	25.0 ± 0.5	0.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	0.0 ± 0.0
6	62.50	45.0 ± 0.0	45.0 ± 0.0	10.0 ± 0.0	15.0 ± 0.5	20.0 ± 0.5	0.0 ± 0.0
7	125.00	55.0 ± 0.5	55.0 ± 0.0	15.0 ± 0.0	30.0 ± 0.5	40.0 ± 0.5	0.0 ± 0.0
8	250.00	75.0 ± 0.5	75.0 ± 0.0	25.0 ± 0.5	55.0 ± 0.5	65.0 ± 0.5	0.0 ± 0.0
9	500.00	100.0 ± 0.0	85.0 ± 0.0	55.0 ± 0.5	80.0 ± 0.0	80.0 ± 0.0	0.0 ± 0.0
10	1000.00	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	90.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0

TABLE-2
RELATIVE TOXICITY OF FIVE ESSENTIAL OILS AGAINST *Cx. quinquefasciatus* (L.) AFTER 24 H OF TREATMENT

Essential oils	LC_{50} (ppm)	95 % Confidence limits (ppm)	Fit of Probit lines			
		LCL-UCL	Slope ± SE	X^2	df	P
Turpentine	63.8	402.530-1558.19	1.58 ± 0.18	8.31	6, 3	P < 0.0001
P. harmala	85.91	533.45-2171.53	1.58 ± 0.18	3.22	7, 2	P < 0.0001
C. nucifera	338.58	974.461-3502.82	2.49 ± 0.39	7.96	5, 4	P < 0.0001
A. indica	207.56	905.52-3805.24	1.87 ± 0.25	1.68	6, 3	P < 0.0001
E. citriodora	165.51	554.68-1704.06	2.30 ± 0.31	1.89	6, 3	P < 0.0001

was 0, 0, 0, 0, 0, 10, 15, 25, 55 and 100 % at concentrations of 1.95, 3.90, 7.81, 15,62, 31.25, 62.50, 125.00, 250.00, 500.00 and 1000.00 ppm, respectively. The LC_{50} value (Table-2) for *Cx. quinquefasciatus* (Say) was 338.58 with lower and upper 95 % confidence limits as 974.461 and 3502.82 respectively. Analysis of variance revealed that concentrations of *C. nucifera* oil tested against *Cx. quinquefasciatus* (Say) were significantly different among all treatments ($F = 1315.37$, $df = 5,4$; $p < 0.0001$).

***Azadirachta indica* oil treatment:** Table-1 revealed the percentage mortality of larvae of *Cx. quinquefasciatus* (Say) when exposed to different concentrations of *A. indica* oil. The % mortality for *Cx. quinquefasciatus* (Say) after 24 h of treatment was 0, 0, 0, 5, 5, 15, 30, 55, 80 and 90 % at concentrations of 1.95, 3.90, 7.81, 15,62, 31.25, 62.50, 125.00, 250.00, 500.00 and 1000.00 ppm, respectively. The LC_{50} value for *Cx. quinquefasciatus* (Say) was 207.56 with lower and upper 95 % confidence limits as 905.52 and 3805.24, respectively. Analysis of variance revealed that concentrations of *A. indica* oil tested against *Cx. quinquefasciatus* (Say) were significantly different among all treatments ($F = 336.63$, $df = 6,3$; $p < 0.0001$).

***Eucalyptus citriodora* oil treatment:** Similarly from Table-1, it was revealed that percentage mortality of larvae of *Cx. quinquefasciatus* (Say) when exposed to different concentrations of *E. citriodora* oil. The % mortality for *Cx. quinquefasciatus* (Say) after 24 h of treatment was 0, 0, 0, 0, 5, 20, 40, 65, 80 and 100 % at concentrations of 1.95, 3.90, 7.81, 15,62, 31.25, 62.50, 125.00, 250.00, 500.00 and 1000.00 ppm, respectively. The LC_{50} value (Table-2) for *Cx. quinquefasciatus* (Say) was 165.51 with lower and upper 95 % confidence limits as 554.68 and 1704.06, respectively. Analysis of variance revealed that concentrations of *E. citriodora* oil tested against *Cx. quinquefasciatus* (Say) were significantly different among all treatments ($F = 4258.53$, $df = 6,3$; $p < 0.0001$).

From results, it was revealed that in control unit all larvae survived even at the end of the experiment.

All the essential oils tested demonstrated significant larvicidal activity on *Cx. quinquefasciatus* (Say), with LC_{50} values ranging from 63.8 to 338.58 ppm. The exposure time is very important in determining the LC_{50} values in tested oils. Results (Table-1) showed that all oils give 100 % mortality at 1000 ppm except *A. indica* which showed 90 % mortality at 1000 ppm. As far as the effectiveness if oil is concerned, the results are significantly different for different essential oils. As seen from the Table-1 *Turpentine* oil was considered highly toxic as it was capable of killing 100 % larvae of *Cx. quinquefasciatus* after 24 h of treatment at concentration of 1000 ppm. Table-1 shows efficacy of *Turpentine* oil against *Cx. quinquefasciatus* (Say) at different concentrations and have LC_{50} value 63.8. After *Turpentine* next effective oil was *P. harmala*. The LC_{50} value for *Cx. quinquefasciatus* is 85.91. As compared to *P. harmala*, *E. citriodora* has a greater LC_{50} value (165.51). More concentration of *Eucalyptus* is required to kill 50 % of population as compared to *P. harmala* oil. Regarding *A. indica* and *C. nucifera*, they showed some delayed toxicity but effective with LC_{50} values 207.56 and 338.58, respectively. All the essential oils selected showed larvicidal effectiveness. Nevertheless upon a comparison of lethal concentrations as determining factor in the larvicidal effectiveness of essential oils, it is apparent that their biological effectiveness differs. So, according to the larvicidal activity of essential oils against *Cx. quinquefasciatus* (Say), the oils were arranged in the following ascending order of preference *i.e.* ***Tupentine > P. harmala > E. citriodora > A. indica > C. nucifera***

Various studies also suggested that plant oils are effective alternative against pests. Abbassi *et al.*[11] demonstrated toxicity of the seed extract on different mosquito species. Comparing total mortality percentages of *P. harmala* acetonic seed extract on different treatments gives a good insight about its bioactivity.

The present work suggests, however, that products based on these essential oils may contribute greatly to a reduction in environment chemicalisation and to an overall reduction of the population density of some significant vectors such as *Cx. quinquefasciatus* (Say). Supplementary investigations for the mode of constituents actions, effects on non-target organisms and old evaluation are necessary. These results obtain in the current study are useful in th discovery of more selective, biodegradable and naturally produced larvicidal compounds.

Conclusion

Essential oils from *E. citriodora*, *P. harmala*, *A. indica*, *C. nucifera* and *Turpentine* were tested against mosquito species *Cx. quinquefasciatus* (Say) to develop biopesticide as an alternative to chemical insecticides. From the results it was concluded that turpentine has highest larvicidal properties. All the essential oils produced gave significant mortality and give 100 % mortality at 1000 ppm except neem oil which gives 90 % mortality at 1000 ppm. This study also revealed that all the essential oils used in the study have remarkable larvicidal properties. Present findings suggest that essential oils may be explored as a potential environmental benign larvicide.

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