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Larvicidal activity of four Spice plant Essential oils against *Culex quinquefasciatus* Say, 1823 (Insecta: Diptera: Culicidae)

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Abstract
A study was conducted to assess the larvicidal potential of spice essential oils from *Piper nigrum* (Black pepper), *Murraya koenigii* (Curry leaf), *Allium sativum* (Garlic) and *Allium cepa* (Onion) against *Culex quinquefasciatus* mosquito larvae under laboratory condition. Various dilutions (12.5ppm, 25ppm, 50ppm, 75ppm, 100ppm, 150 ppm, 200ppm, 250ppm, 500ppm) of commercially available steam distilled essential oil used to carry out laboratory bioassays. The percentage of mortality of *Culex quinquefasciatus* was calculated after 24, 48, 72 and 96 hours of exposure. Among the essential oils tested Garlic, showed the highest larvicidal activity against larvae of *Culex quinquefasciatus* (LC_{50} 0.258 ppm and LC_{90} 0.335 ppm) on 24 hours of exposure. Black pepper, Curry leaf and Onion also showed significant amount of larvicidal activity (LC_{50} 0.288 ppm, 0.385 ppm, 0.361 ppm and LC_{90} 0.471, 0.443, 0.416 respectively). As all the essential oils show significant larvicidal effect, it can be concluded that essential oils can combat *Culex quinquefasciatus* larvae. And thus, these compounds may provide an alternative to synthetic insecticides as they are environmentally safe insecticides.

Keywords: Essential oils, *Culex quinquefasciatus*, Larvicidal activity, LC_{50}, LC_{90}

Introduction

Mosquitoes transmit deadly diseases, causing lots of human deaths every year globally. *Culex quinquefasciatus* Say, 1823 is a vector of lymphatic filariasis, which is broadly distributed tropical disease. Every year, large number of peoplefound to get infectedwith this disease worldwide and most of them shows common chronic manifestation [7].

Theeradication of the disease can be managed by either targeting mosquito larvae through spraying stagnant water breeding sites or by eliminating adult mosquitoes, using insecticides [6]. Larvicide is an effective method for reducing mosquito populations in their breeding places before they emerge as adult. However, the use of pesticides in water induces significant risks to both people and the environment. Many of these pesticides, such as pyrethroids, are highly toxic to fish and should not be used in ecosystem containing fish or crustaceans.The frequent use of synthetic insecticides leads to the disruption of ecosystems and increases the resistance of pests to the insecticides [8].Recently, environmentally friendly and biodegradable natural insecticides of plant origin have gained attention as an alternative green measure for controlling arthropods of public health importance [9].

Natural pesticides derived from plants are highly effective against mosquitoes. The chemical compounds extracted from plant materials can serve as repellents, Larvicide, oviposition attachments, insect growth regulators and deterrent agents [4]. Plant products have been used worldwide for killing or repelling mosquitoes, either as extracts or as whole. Certain natural products have been investigated for the repellent activity against mosquitoes, extracts of several plants, including neem (*Azadirachta indica*), clove (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) have been studied as potential mosquito repellents [2, 3, 11, 13].

The present research was designed to evaluate the Larvicidal activity of four essential oilsi.e, *Piper nigrum*, *Murraya koenigii*, *Allium sativum* and *Allium cepa* against the mosquito larvae of *Culex quinquefasciatus.*

Material and Methods

Collection of mosquito larvae

All tests were conducted in Applied Entomology laboratory, Department of Zoology, Vidyasagar college, Saltlake campus. The larvae of *Culex* spp. Mosquito were collected from drains surrounding areas of Vidyasagar College campus and were brought into the laboratory. *Culex quinquefasciatus* mosquito larvae (Figure: 3, A).were identified by their morphological characters, then the larvae were kept separately in an aquarium using optimum conditions.

Essential Oils

To access the larvicidal activity of essential oils commercially available steam distilled essential oils of *Piper nigrum* L. (Black pepper), *Murraya koenigii* L. (Curry leaf), *Allium sativum* L. (Garlic) and *Allium cepa* L. (Onion) were collected from local market.

Evaluation of essential oils with respect to their larvicidal properties

Three replicates of each oil were prepared by dissolving suitable amount of essential oil stock solution in 100ml of distilled water. Stock solution was prepared by dissolving 1 ml of essential oil in 2ml of 100% acetone followed by 1000ml of distilled water. The stock solution was used to prepare serial dilutions of target oil in the concentration of 12.5, 25, 50, 75, 100, 150, 200, 250, 500 ppm through the dilution of stock solution with distilled water. While controlled replicant was prepared with 0 ppm of oil, 2 ml of acetone and 198ml of distilled water. [5].

Bioassay of oil solution

Each ppm concentration prepared were divided into 3 plastic cups, containing the same amount of liquid. 10 *Culex*

quinquefasciatus larvae were placed in each cup, after that the cups were labelled as R_1, R_2 and R_3 with following concentration, and were placed in large trays. The trays were then covered with newspaper filled with some holes for proper ventilation. The cups were observed after 24, 48, 72 and 96 hours. The number of dead larvae were counted after 24, 48, 72, 96 hours and noted down respectively.

Calculation of LC_{50}, LC_{90}, and statistical analysis

Mortality percentages were calculated followed by probit analysis with the help of regression in MS Word. LC_{50} (the concentration at which 50% of larvae were immobilized) and LC_{90} (the concentration at which 90% of larvae were immobilized) values were calculated by using Finney's probit table followed by log formula of MS Word.

Results

The percentage mortality of mosquito species *Culex quinquefasciatus* larvae when exposed to different concentrations of oils i.e., *Piper nigrum*, *Murraya koenigii*, *Allium sativum* and *Allium cepa*after 24, 48, 72 and 96 hours of exposure are shown in Table-1, Table-2, Table-3, and Table-4 respectively. Table-5 shows relative toxicity of the said essential oils.

Treatment with *Piper nigrum* oil: It was revealed from Table: 1 that percentage mortality for larvae of *C. quinquefasciatus* when exposed to different concentrations of *Piper nigrum* oil after 24 hours of treatment was 16, 20, 43, 46, 46, 60, 73, 76, and 80% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. Similarly, in Table-2 it is shown the percent mortality of *C. quinquefasciatus* after 48 hours of treatment were 26, 40, 53, 53, 60, 63, 76, 80 and 90% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. In Table-3 it is shown the percent mortality of *C. quinquefasciatus* after 72 hours of treatment were 40, 50, 66, 70, 73, 76, 83, 93, and 100% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. In Table-4 it is shown the percent mortality of *C. quinquefasciatus* after 96 hours of treatment were 50, 56, 70, 76, 76, 83, 86, 100, and 100% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. It can be decided from all the tables that the larval mortality increases with the increase of concentrations. No mortality was observed in the control medium after 96 hours of test period. The LC_{50} Value for *C. quinquefasciatus* larvae after 24, 48, 72 and 96 hours were 0.288, 0.299, 0.176 and 0.149 respectively. The LC_{90} Value for *C. quinquefasciatus* larvae after 24, 48, 72 and 96 hours were 0.470, 0.451, 0.328 and 0.287 respectively (Table: 1-4; Figure: 1-2). The larvae turned various shades of green after treatment with this oil (Figure: 3, B).

Treatment with *Murraya koenigii* oil: In the same way from Table: 1 that percentage mortality for larvae of *C. quinquefasciatus* when exposed to different concentrations of *Murraya koenigii* oil after 24 hours of treatment was 0, 0, 6, 10, 13, 16, 26, 30, and 76% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. Similarly, in Table-2 it is shown the percent mortality of *C. quinquefasciatus* after 48 hours of treatment were 10, 13, 16, 23, 33, 40, 43, 50 and 86% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. In Table-3 it is shown the percent mortality of *C. quinquefasciatus* larvae after 72 hours of treatment were 13, 30, 33, 36, 50, 56, 60, 70, and 96% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. In Table-4 it is shown the percent mortality of *C. quinquefasciatus* larvae after 96 hours of treatment were 26, 40, 43, 46, 63, 66, 80, 83 and 100% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. It can be decided from all the tables that the larval mortality increases with the increase of concentrations. No mortality was observed in the control medium after 96 hours of test period. The LC_{50} Value for larvae of *C. quinquefasciatus* after 24, 48, 72 and 96 hours were 0.385, 0.359, 0.288 and 0.228 respectively. The LC_{90} Value for *C. quinquefasciatus* larvae after 24, 48, 72 and 96 hours were 0.443, 0.511, 0.447 and 0.356 respectively (Table: 1-4; Figure:1-2).The larvae turned whitish in color after treatment with this oil (Figure: 3, C).

Treatment with *Allium sativum* oil: In the same way from Table: 1 that percentage mortality for larvae of *C. quinquefasciatus* when exposed to different concentrations of *Allium sativum*oil after 24 hours of treatment was 6, 10, 13, 36, 50, 63, 83, 100 and 100% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. Similarly, in Table-2 it is shown the percent mortality of *C. quinquefasciatus* larvae after 48 hours of treatment were 10, 16, 56, 70, 80, 93, 93, 100 and 100% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. In Table-3 it is shown the percent larval mortality of *C.quinquefasciatus* after 72 hours of treatment were 13, 30, 70, 80, 86, 96, 100, 100, and 100% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. In Table-4 it is shown the percent larval mortality of *C. quinquefasciatus* after 96 hours of treatment were 36, 50, 83, 96, 100, 100, 100, 100 and 100% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. It can be decided from all the tables that the larval mortality increases with the increase of concentrations. No mortality was observed in the control medium after 96 hours of test period. The LC_{50} Value for the larvae of *C. quinquefasciatus* after 24, 48, 72 and 96 hours were 0.258, 0.214, 0.183 and 0.092 respectively. The LC_{90} Value for *C. quinquefasciatus* larvae after 24, 48, 72 and 96 hours were 0.335, 0.302, 0.272 and 0.207 respectively (Table: 1-4; Figure:1-2).The larvae turned various shades of green after treatment with this oil (Figure: 3, D).

Treatment with *Allium cepa* oil: In the same way from Table: 1 that percentage mortality for larvae of *Culex quinquefasciatus* when exposed to different concentrations of *Allium cepa*oil after 24 hours of treatment was 0, 0, 3, 10, 20, 56, 60, 66, and 73% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. Similarly, in Table-2 it is shown the percent larval mortality of *C.quinquefasciatus* after 48 hours of treatment were 0, 0, 6, 13, 26, 60, 66, 70, and 76% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. In Table-3 it is shown the percent larval mortality of *C. quinquefasciatus* after 72 hours of treatment were 13, 20, 26, 36, 36, 76, 83, 86, and 96% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. In Table-4 it is shown the percent larval mortality of *C. quinquefasciatus* after 96 hours of treatment were 16, 23,

36, 43, 53, 83, 86, 90, and 100% at concentration of 12.5, 25, 50, 75, 100, 150, 200, 250 and 500 ppm, respectively. It can be decided from all the tables that the larval mortality increases with the increase of concentrations. No mortality was observed in the control medium after 96 hours of test period. The LC_{50} Value for *C.quinquefasciatus* larvae after 24, 48, 72 and 96 hours were 0.361, 0.354, 0.277 and 0.242 respectively. The LC_{90} Value for the larvae of *C. quinquefasciatus* after 24, 48, 72 and 96 hours were 0.416, 0.409, 0.409, and 0.348 respectively.. (Table: 1-4; Figure:1-2). The larvae showed damage in thorax and tail after treatment with this oil (Figure: 3, E-F).

Table 1: Percentage mortality ± SE of *Culex quinquefasciatus* larvae when exposed to different concentration of four spice essential oils and controlled medium after 24 hours of exposure

Concentrations (ppm)	Mortality(%) ± SE				Control
	***Piper nigrum*oil**	***Murrayakoenigii*oil**	***Allium sativum*oil**	***Allium cepa* oil**	
12.5	16±0.33	0±0	6±0.33	0±0	0±0
25	20±0	0±0	10±0	0±0	0±0
50	43±0.88	6±0.33	13±0.88	3±0.33	0±0
75	46±0.33	10±0.57	36±0.33	10±0.57	0±0
100	46±0.33	13±0.33	50±0.57	20±0.57	0±0
150	60±0.57	16±0.88	63±0.33	56±0.33	0±0
200	73±0.66	26±0.66	83±0.33	60±0.57	0±0
250	76±0.33	30±0.33	100±0	66±0.88	0±0
500	80±0	76±0.66	100±0	73±0.66	0±0

Table 2: Percentage mortality ± SE of *Culex quinquefasciatus* larvae when exposed to different concentration of four spice essential oils and controlled medium after 48 hours of exposure

Concentrations (ppm)	Mortality(%) ± SE				Control
	***Piper nigrum* oil**	***Murraya koenigii* oil**	***Allium sativum* oil**	***Allium cepa* oil**	
12.5	26±0.33	10±0	10±0	0±0	0±0
25	40±0.57	13±0.33	16±0.33	0±0	0±0
50	53±0.33	16±0.33	56±1.20	6±0.33	0±0
75	53±0.33	23±0.33	70±0.57	13±0.33	0±0
100	60±0	33±0.88	80±0	26±0.33	0±0
150	63±0.33	40±0.57	93±0.66	60±0	0±0
200	76±0.33	43±0.88	93±0.33	66±0.33	0±0
250	80±0	50±0.57	100±0	70±0.57	0±0
500	90±0	86±0.33	100±0	76±0.33	0±0

Table 3: Percentage mortality ± SE of *Culex quinquefasciatus* larvae when exposed to different concentration of four spice essential oils and controlled medium after 72 hours of exposure

Concentrations (ppm)	Mortality(%) ± SE				Control
	***Piper nigrum* oil**	***Murraya koenigii* oil**	***Allium sativum* oil**	***Allium cepa* oil**	
12.5	40±0	13±0	13±0.33	13±0.33	0±0
25	50±0	30±0.33	30±0.57	20±0.57	0±0
50	66±0.33	33±0	70±1.73	26±0.33	0±0
75	70±0	36±0.33	80±0.33	36±0.33	0±0
100	73±0.33	50±0.33	86±0.33	36±0.33	0±0
150	76±0.33	56±0.66	96±0.33	76±0.33	0±0
200	83±0.33	60±0.57	100±0	83±0.33	0±0
250	93±0.33	70±0.57	100±0	86±0.33	0±0
500	100±0	96±0.33	100±0	96±0.33	0±0

Table 4: Percentage larval mortality ± SE of *Culex quinquefasciatus* when exposed to different concentration of four spice essential oils and controlled medium after 96 hours of exposure

Concentrations (ppm)	Mortality(%) ± SE				Control
	***Piper nigrum* oil**	***Murraya koenigii* oil**	***Allium sativum* oil**	***Allium cepa* oil**	
12.5	50±0	26±0.33	36±0.88	16±0.33	0±0
25	56±0.33	40±0	50±0.57	23±0.33	0±0
50	70±0.57	43±0.33	83±1.20	36±0.33	0±0
75	76±0.33	46±0.33	96±0.33	43±0.33	0±0
100	76±0.33	63±0.33	100±0	53±0.33	0±0
150	83±0.33	66±0.33	100±0	83±0.33	0±0
200	86±0.33	80±0.57	100±0	86±0.33	0±0
250	100±0	83±0.33	100±0	90±0	0±0
500	100±0	100±0	100±0	100±0	0±0

Table 5: Log-Probit analysis and Regression analysis of Larvicidal activity of *Piper nigrum*, *Murraya koenigii*, *Allium sativum* and *Allium cepa* after 24, 48, 72 and 96 hours of exposure on *Culex quinquefasciatus*

Oil used	Time	Regression equation	r^2 value	LC_{50} (ppm)	LC_{90} (ppm)	P value	95% confidence limits (ppm)
							LCL-UCL
Piper nigrum	24 hrs	y = 1.2615x + 2.5493	0.960	0.288	0.470	3.60876E-06	2.087-3.011
	48 hrs	y = 1.129x + 3.0847	0.960	0.229	0.451	4.75537E-07	2.669-3.499
	72 hrs	y = 2.0262x + 1.9591	0.682	0.176	0.328	0.1055	-0.534-4.452
	96 hrs	y = 2.4362x + 1.5598	0.653	0.149	0.287	0.2868	-1.64-4.759
Murrayakoenigii	24 hrs	y = 3.661x - 3.888	0.877	0.385	0.443	0.0073	-6.351-(-)1.4244
	48 hrs	y = 1.3379x + 1.9406	0.872	0.359	0.511	0.0016	1.019-2.862
	72 hrs	y = 1.4968x + 2.0902	0.864	0.288	0.447	0.0024	1.022-3.158
	96 hrs	y = 2.1926x + 1.2918	0.659	0.228	0.356	0.3182	-1.550-4.134
Allium sativum	24 hrs	y = 3.6423x - 1.6	0.771	0.258	0.335	0.3245	-5.170-1.970
	48 hrs	y = 3.4736x - 0.6872	0.882	0.214	0.302	0.4996	-2.970-1.596
	72 hrs	y = 3.6657x - 0.587	0.896	0.183	0.272	0.5557	-2.830-1.656
	96 hrs	y = 3.3916x + 0.8057	0.840	0.092	0.207	0.4970	-1.854-3.465
Allium cepa	24 hrs.	y = 4.1418x - 4.517	0.898	0.361	0.416	0.0037	-7.026-(-)2.008
	48 hrs	y = 4.1943x - 4.4921	0.883	0.354	0.409	0.0060	-7.233-(-)-1.750
	72 hrs	y = 1.9002x + 1.4039	0.904	0.277	0.409	0.0203	0.291-2.516
	96 hrs	y = 2.6656x + 0.3378	0.798	0.242	0.348	0.7506	-2.079-2.754

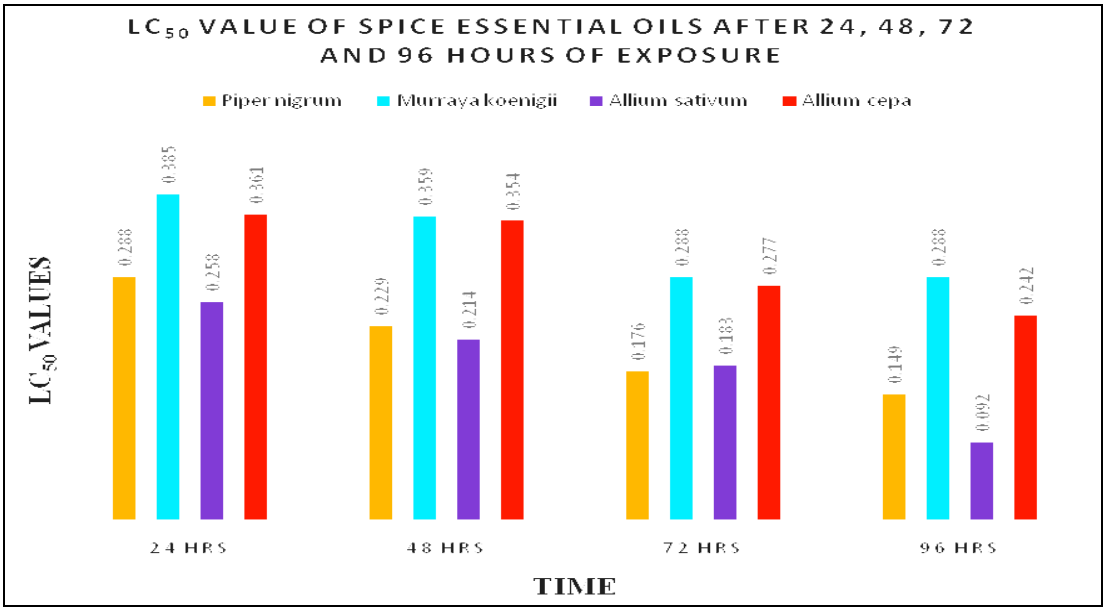

Fig 1: Graph showing LC_{50} values of four essential oils at different time intervals

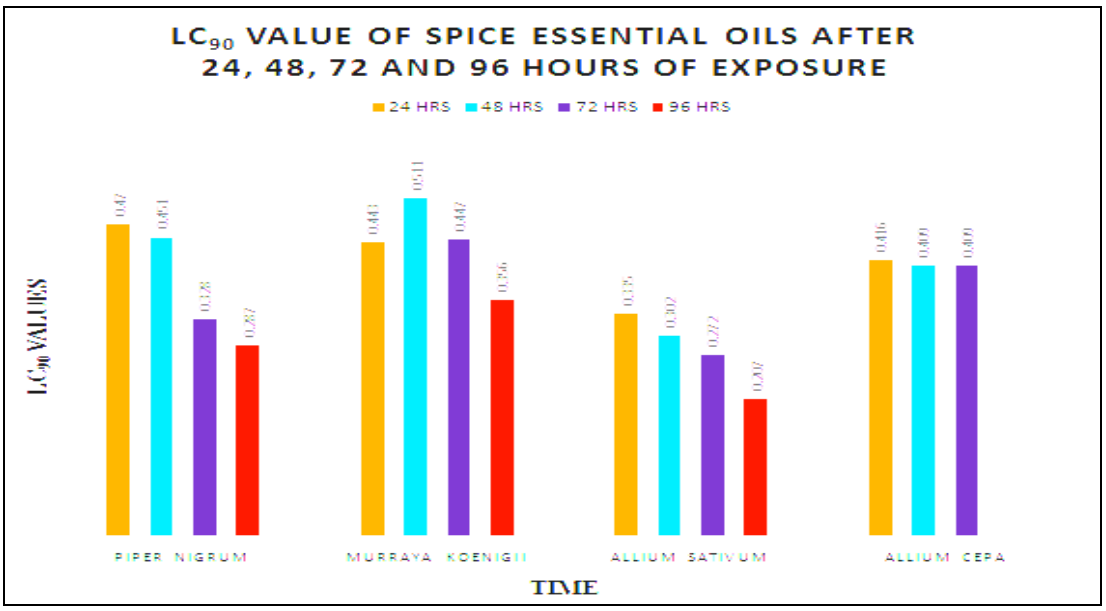

Fig 2: Graph showing LC_{90} values of four essential oils at different time intervals

Fig 3: Morphological deformities of *Culex quinquefasciatus* mosquito larvae after treatment with Essential oils. A) Normal larvae, B) larvae after treatment with *Piper nigrum*, C)larvae after treatment with *Murraya koenigii*, D) larvae after treatment with *Allium sativum*, E)& F) larvae after treatment with *Allium cepa.*

Discussion

During the present study, the essential oil of *Allium sativum* showed the highest larvicidal activity against larvae of *C. quinquefasciatus* (LC_{50} 0.258 ppm and LC_{90} 0.335 ppm) on 24 hours of exposure followed by *Piper nigrum*, *Murraya koenigii* and *Allium cepa* (LC_{50} 0.288 ppm, 0.385 ppm, 0.361 ppm and LC_{90} 0.471, 0.443, 0.416 respectively).

Zhu *et al.*, [14] conducted an experiment on *Aedes albopictus*, *Aedes aegypti*, and *Culex pipiens pallens* mosquitoes using five different essential oils. Thyme, catnip, Amyris, eucalyptus and lemon oils were used in the experiment. Dimethyl sulfoxide was used as solvent. The concentrations prepared were 40, 80, 160 and 320 µg/ml. Among the oils used Amyris oil demonstrated the greatest larvicidal effects on the mosquitoes with the LC_{50} values of 58 µg/ml for *A. aegypti*, 78 µg/ml for Ae. albopictus, and 77 µg/ml for C p.pallens after 24 hours of exposure.

Abdelkrim *et al.*, [1] conducted an experiment on *Aedes*, *Anopheles* and *Culex* larvae, using 41 different plants to extract 13 different essential oils (camphor, thyme, amyris, lemon, cedarwood, frankincense, dill, myrtle, juniper, black pepper, verbena, helichrysum, and sandalwood). The concentrations prepared were 1, 10, 50, 100 and 500 ppm. The LC_{50} values of these oils ranged between 1 to 101.3 ppm against A. aegypti, between 9.7 and 101.4 ppm for A. stephensi and between 1 and 50.2 ppm for *C. quinquefasciatus*.

Sritabutra and Soonwera [12] worked on the repellent activity of herbal essential oils against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* and concluded that clove, citronella and lemongrass oil were the most promising for repellency against mosquito species.

Pavela [10] tested essential oils from 22 aromatic plant species for mortality of the mosquito larvae *Culex quinquefasciatus* and observed that the lethal Essential oils obtained from *Thymus vulgaris*, *Satureja hortensis* and *Thymus satureioides* plants showed the highest effect, with LC_{50} found lower than 50 µg/ml (33, 36 and 44 µg/ml, respectively).

Conclusion

Essential oils from *Piper nigrum*, *Murraya koenigii*, *Allium sativum* and *Allium cepa* were tested against *Culex quinquefasciatus* to determine if plant extracted essential oils can work as insecticides. In conclusion the study revealed that all the essential oils have larvicidal properties. India being gifted with diverse range of spice and herb plant, can be helpful for experiments like these in the future.

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