ADULTICIDAL ACTIVITY OF BOTANICAL OILS BY IMPREGNATED PAPER ASSAY AGAINST CULEX QUINQUEFASCIATUS SAY

M. RAMAR1,2, S. IGNACIMUTHU1, P. MANONMANI1, K. MURUGAN3
1Entomology Research Institute, Loyola College, Chennai-34, Tamil Nadu, India, 2Department of Biotechnology, PRIST University, Vallam, Thanjavur, 3Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar, University, Coimbatore, Tamil Nadu 641046, India
Email: ramareri@gmail.com

Received: 19 Oct 2016 Revised and Accepted: 31 Mar 2017

Abstract

Objective: The present study was undertaken with the aim of finding out the efficacy of essential oils (EOs) as anti-mosquito agents for commercial purposes. Plant source insecticides as an alternative to chemical insecticide, this study was evaluated to assess the knock-down and adulticidal prospective of the essential oils against Culex quinquefasciatus. The plant essential oil is largely cultivated throughout India and in all Tropical countries.

Methods: The selected botanical essential oils were procured from commercial producers of plant essential oils and aromatic substances were used in this study. Knock-down and Adulticidal bioassay was performed according to WHO protocol. A single dose of the essential oils was used in the preliminary screening. 20 adult mosquitoes (3-5 d old glucose fed mosquitoes) were exposed on treated paper for one hour and knocked down and live mosquitoes were counted at 5 minute intervals.

Results: Among the twenty three oils tested, 100% knock-down and adult mortality was recorded at 10% /cm² of clove oil, camphor, cinnamon, citronella, clove, eucalyptus, lemongrass, pine, thyme and tuli oils respectively. At 10% concentration, clove oil (K90 = 1.8 %/cm²) and KT95 = 2.03 min) was found to be the most potential treatment. After 15 min exposure period clove oil registered the lowest knock-down dose which was calculated as (K90 = 1.8 %/cm²) and (K90 = 1.2 %/cm²). The lower and upper 95% confidence limits for clove oil were calculated as 0.2 and 4.2 min respectively.

Conclusion: From the results it can be concluded that the adult of the Cx. quinquefasciatus were susceptible to the essential oils. Such findings would be useful in promoting research aiming at the development of new agent for mosquito control on basis of chemical compounds from indigenous plant sources as an alternative to chemicals.

Keywords: Essential oils, Screening, Filaria Mosquito, Culex quinquefasciatus, Knock-down-Adulticidal

INTRODUCTION

Mosquito diseases, such as malaria, Japanese encephalitis, filariasis, dengue and yellow fever a major source of illness and death worldwide, particularly in tropical and subtropical area [1]. The Culex quinquefasciatus is a major public health problems in India and filariasis is potentially risk of infection [2]. These diseases affect the health and quality of life of millions of people in Worldwide [3]. An estimated 120 million people in tropical and subtropical areas of the world are infected with lymphatic filariasis [4]. Moreover, mosquitoes cause allergic responses in humans that include local skin and systemic reactions [5].

Such serious diseases are becoming increasingly difficult because of the high rate of reproduction and development of resistance to insecticides in mosquitoes [6]. The use of chemical control of mosquitoes is remaining as a main part of integrated vector management [7-8]. According to Word Health Organization pesticide, the major insecticides used against mosquitoes are pyrethroids and organophosphates [7-8]. Although, factors including the development of resistance to insecticides are leading to morbidity and mortality due to malaria and other vector-borne diseases. Insect resistance has been reported to chemical class of insecticide used in vector control programs and insect growth regulators [9].

The search for alternative pesticides and control measures that pose no risk or posing minimal risk to human health and the environment is of great interest from the preventive medicine point of view [10]. Pyrethrin-based mosquito liquid formulations are widely used in many countries, especially in the house hold of rural population. Interest in botanical pesticides revived during recent years, because of the deleterious effects of synthetic insecticides, including lack of selectivity, impact on the environment and the emergence and spread of pest resistance. The naturally occurring pesticides appear to have a promising role in the development of future commercial pesticides for safety of the environment and public health [11-12].

Essential oils from plant species, celery (Apium graveolens), caraway (Carum carvi), zedoary (Curcuma zedoaria), long pepper (Piper longum) and Chinese star anise (Illicium verum), were studied adulticidal activity against mosquito vectors [13]. Adulticidal activity of five essential oils such as Citrus sinensis, Mentha pipreta, Corvocrt oil, Citronela oil and citral oil against Cx. Quinquefasciatus [14]. The fumigant toxicity of essential oils from 15 species of African plants against Anopheles gambiense in the laboratory conditions [15].

Plant essential oils are volatile substances found in a various plants. This oil isolated from plant source, but thus oils are consisting of mixtures of many compounds. Botanical based oils were the first preservatives used by man, their natural state within plant tissues and as oils obtained by water distillation. The oils composed by isoprenoid compounds, mainly mono- and sesquiterpenes are the carriers of the smell found in aromatic plants [16]. The uses of commercial, essential oils are used in four primary ways: pharmaceuticals, flavor enhancers in many food products, odourants in fragrances and insecticides.

At present, evaluation of essential oils against mosquitoes and isolation, identification and development of natural products from them are under the focus of numerous research programmes around the globe. There is a renewed interest in plant essential oils products as sources of new insect controlling agents, because they may be biodegradable to nontoxic compounds, thus minimizing the accumulation of harmful residues, leading them to be more environmentally friendly compared to synthetic compounds [17].
The present study was undertaken to evaluate the bio-efficacy of some essential oils (EOs) against Culex quinquefasciatus adults mosquito and to study effects of the effective oils on adults of the mosquito.

**MATERIALS AND METHODS**

**Materials**

**Botanical essential oils**

For the present study, twenty two botanical essential oils were procured from Tegraj and Co (P) Ltd, India (A commercial producer of plant essential oils and aromatic sources) were used in this study. The essential oils (EOs) were selected primarily on the basis of ethno-botanical information and various biological effects, the following 22 essential oils (EOs) were selected: Aniseed (Pimpinella anisum Linn.), Bergamot (Citrus bergamia Risso), Calamus (Acorus calamus L.), Camphor (Cinnamomum camphora Linn.), Cedarwood (Cedrus atlantica Endl), Cinnamon (Cinnamomum verum J. S. Presl), Citronella (Cymbopogon nardus Linn.), Clove (Myrtus caryophyllus Linn.), Eucalyptus (Eucalyptus globulus Labill), Geranium (Pelargonium graveolens L.), Lemon (Citrus limon Linn.), Lemongrass (Cymbopogon flexuosus J. F. Watson), Lime (Citrus aurantiifolia Christm.), Luchi (Gaultheria fragrantissima Cham), Nutmeg (Myristica fragrans Houtt.), Orange (Citrus sinensis Linn.), Palmarosa (Cymbopogon martin Stapf), Pine (Pinus radiata D. Don), Rosemary (Rosmarinus officinalis Linn.), Thyme (Thymus vulgaris Linn.), Tulsi (Ocimum sanctum Linn.) and Vetiver (Vetiveria zizanioides Linn.). The essential oils were stored in the refrigerator until used for bioassay experiments.

**Maintenance of test organism**

The study organism of Cx. quinquefasciatus was maintained in several generations at Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India. Mosquitoes were free of exposure to pathogens and insecticides and maintained at 26±1 °C and 60-80% relative humidity (RH). Larvae were fed on dog biscuits and yeast powder in a ratio 3:2 until molting to become pupae. pupae was transferred into a mosquito rearing cage. The pupae were transferred from culture trays to glass beakers containing tap water and placed in cages (45 x45 x 45 cm), where adults emerged. The cages was made up of metal and covered with a muslin cloth. The emergent adults were fed with 10% glucose solution dipped in cotton.

**Methods**

**Knock-down and adulticidal activity**

The Knock-down and killing bioassay was performed according to WHO protocol [18]. The selected essential oil was prepared in 2 ml of acetone. A single dose of the oil (10%) was used in the preliminary screening. Each essential oil was prepared in 2 ml of acetone and applied on Whitman no. 1 filter papers (size 12 x15 cm). control papers were treated with 2 ml of acetone alone and placed in exposure tubes. 3-5 d old sugar fed mosquitoes (In each tube, 20 adult mosquitoes) were exposed on treated paper for one hour and knocked down and live mosquitoes were recorded at every 5 minute intervals. After one-hour exposure mosquitoes was transferred into recovery test tubes for 24 hour mortality observation. Five replicates were run at a time with control. The reference insecticide was used with 0.5% deltamethrin impregnated paper as a comparison. The median knock-down time (KT50 and KT90) and knock-down dose (KD50 and KD90) were calculated by Probit analysis.

\[
\text{Adult Knockdown (\%) = \frac{\text{Number of adults}}{\text{Number of adults released}}} \times 100
\]

**Data analysis**

Statistical analysis was performed using SPSS software package, version 15. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT)[19]. The median Knock down time (KT50 and KT90) and lethal dose (KD50 and KD90) was carried out by Probit analysis [20]. p value of<0.05 was considered to represent Significant differences. The corrected percent mortality was evaluated by using Abbott’s formula [21].

\[
\text{Corrected mortality (\%) = } \frac{\text{Mortality in control (\%) - Mortality in treatment (\%) \times 100}}{100 - \text{Mortality in control (\%)}}
\]

**RESULTS**

**Percentage of knock-down and killing effect**

The screening efficacy of Knock-down and killing effect of the plant oil on Culex quinquefasciatus was studied and is illustrated in the table 1. In the present preliminary screening study a single dose (10%) of EOs was used and acetone served as control and reference insecticide (0.05 % deltamethrin) was also used as comparison.

| Plant essential oils       | % knock-down (cm²) at 1 h | Mortality (%) after 24 h |
|----------------------------|--------------------------|-------------------------|
| Aniseed                    | 73.6±0.61 i              | 54.3±1.23 i             |
| Bergamot                   | 60.0±0.52 i              | 36.3±1.21 i             |
| Calamus                    | 100.0±0.00               | 100±0.00                |
| Camphor                    | 100.0±0.00               | 100±0.00                |
| Cedarwood                  | 63.3±0.31 i              | 39.0±0.51 i             |
| Cinnamom                   | 100.0±0.00 a             | 96.6±0.82 a             |
| Citronella                 | 100.0±0.00 a             | 100±0.00                |
| Clove                      | 100.0±0.00               | 100±0.00                |
| Eucalyptus                 | 100.0±0.00               | 100±0.00                |
| Geranium                   | 86.6±0.31 i              | 58.3±0.82 i             |
| Lemon                      | 36.6±0.32 b             | 16.6±0.34 b             |
| Lemongrass                 | 100.0±0.00               | 91.3±2.34 b             |
| Lime                       | 66.3±0.32 i              | 38.6±0.81 i             |
| Luchi                      | 62.0±0.31 i              | 38.0±0.52 i             |
| Nutmeg                     | 53.0±0.32 i              | 27.3±1.22 b             |
| Orange                     | 53.0±0.34 i              | 35.3±0.31 i             |
| Palmarosa                  | 50.0±1.00 c             | 36.6±0.82 c             |
| Pine                       | 100.0±0.00 a             | 100±0.00                |
| Rosemary                   | 73.3±0.32 i              | 62.0±0.53 i             |
| Thyme                      | 100.0±0.00               | 97.6±0.81 i             |
| Tulsi                      | 100.0±0.00               | 96.6±0.82 i             |
| Vetiver                    | 60.0±0.51 i              | 38.6±1.33 i             |
| Deltamethrin (0.05%)       | 100.0±0.00 a             | 100±0.00                |
| Solvent (Acetone)           | 0.0±0.00                 | 3.1±0.24 a              |

Values with the same letter were not significantly different (P<0.05) by one way ANOVA with DMRT.
The results clearly indicated that 100% knock-down and adult mortality was recorded at 10% dose of calamus, camphor, cinnamon, citronella, clove, eucalyptus, lemongrass, pine, thyme and tulu oils respectively. The lowest knock-down and adulticidal activity of 36.6% and 15.4% were recorded in lemon oil. Reference insecticide (0.05% deltamethrin) showed 100% knock-down and adult mortality against Cx. quinquefasciatus.

**Median knock-down time (KT$_{50}$) and KT$_{90}$**

The KT$_{50}$ and KT$_{90}$ of effective essential oils are shown in table 2. At 10% concentration, clove oil (KT$_{50}$=1.8 and KT$_{90}$=2.03 min) was found to be the most potential treatment. It recorded 1.8 min as KT$_{50}$ after 1 h exposure period for citronella (KT$_{50}$=2.5 and KT$_{90}$=28.0 min) and eucalyptus (KT$_{50}$=3.6 and KT$_{90}$=44.3 min) oils were also recorded as potentially effective adulticides against Cx. quinquefasciatus. In 0.05% deltamethrin (KT$_{50}$=2.3 and KT$_{90}$=27.13 min) was registered after 1 hour exposure period.

**Median knock-down dose (KD$_{50}$) and KD$_{90}$**

Ten essential oils were identified as potential treatments in screening bioassay of knock-down and killing activity. Tables 3a and 3b present the median knock-down dose (KD$_{50}$ and KD$_{90}$) of the essential oils against Cx. quinquefasciatus adults at 10% concentration.

After 15 min exposure period clove oil registered the lowest knock-down dose which was calculated as (KD$_{50}$=1.8 %/cm$^2$ and KD$_{90}$=11.2 %/cm$^2$). Next to clove oil citronella (KD$_{50}$=3.0% and KD$_{90}$=11.5%/cm$^2$) and eucalyptus (KD$_{50}$=3.4 and KD$_{90}$=11.6%/cm$^2$) oils were also effective since they recorded lower median knock-down dose.

Tulsi (KD$_{50}$=7.4 and KD$_{90}$=16.4%/cm$^2$) and calamus (KD$_{50}$=8.7 and KD$_{90}$=22.4%/cm$^2$) oils were the least effective knock-down treatment. The lower and upper 95% confidence limits for clove oil were calculated as 0.2 and 4.2 min respectively.

**Table 2: Median knock-down time (KT$_{50}$) and KT$_{90}$ of effective essential oils against Culex quinquefasciatus at 10%/cm$^2$ after 1 hour exposure period**

| Plant essential oils | KT$_{50}$ (min) | 95% CL\textsuperscript{a} | KT$_{90}$ (min) | 95% CL\textsuperscript{a} | Chi-square |
|----------------------|-----------------|-----------------|-----------------|-----------------|------------|
|                      | LCL             | UCL             | LCL             | UCL             |            |
| Calamus              | 22.4            | 19.35           | 34.17           | 44.81           |            |
| Camphor              | 11.0            | 8.324           | 54.58           | 32.7            |            |
| Cinnamon             | 7.6             | 4.322           | 24.45           | 47.6            |            |
| Citronella           | 2.5             | 1.123           | 5.842           | 28.0            |            |
| Clove                | 1.8             | 0.214           | 4.231           | 2.03            |            |
| Eucalyptus           | 3.6             | 1.243           | 15.44           | 44.3            |            |
| Lemongrass           | 20.3            | 16.36           | 23.50           | 44.6            |            |
| Pine                 | 15.4            | 12.44           | 26.34           | 26.3            |            |
| Thyme                | 19.7            | 15.57           | 27.56           | 43.6            |            |
| Tulsi                | 24.4            | 13.24           | 25.23           | 52.3            |            |
| Deltamethrin (0.05%) | 12.0            | 9.310           | 64.59           | 42.6            |            |

Chi-square value significant at P<0.05 level. \textsuperscript{a}CL: confidence limits; LCL: lower confidence limits; UCL: upper confidence limits, KT$_{50}$ = Knock down time required to kill 50% of the population exposed, KT$_{90}$ = Knock down time required to kill 90% of the population exposed.

**Table 3a: Median knock-down dose (KD$_{50}$) and KD$_{90}$ of effective essential oils against female adult Cx. quinquefasciatus at 10%/cm$^2$ after 1 hour exposure period**

| Plant essential oils | Exposure period (min) | KD$_{50}$ (%) | 95% CL\textsuperscript{b} | KD$_{90}$ (%) | 95% CL\textsuperscript{b} | Chi-square |
|----------------------|-----------------------|---------------|-----------------|---------------|-----------------|------------|
|                      |                       | LCL           | UCL             | LCL           | UCL             |            |
| Calamus              | 15                    | 8.7           | *               | 22.4          | *               | 5.8        |
|                      | 30                    | 5.0           | 3.79            | 17.1          | 12.1            | 3.5        |
|                      | 45                    | 1.9           | 0.87            | 12.4          | 9.08            | 2.4        |
| Camphor              | 15                    | 1.8           | -1.03           | 18.0          | 10.4            | 1.5        |
|                      | 30                    | 1.6           | 0.23            | 16.2          | 18.2            | 1.7        |
|                      | 45                    | -1.8          | -6.58           | 9.6           | 6.61            | 2.6        |
| Cinnamon             | 15                    | 4.6           | *               | 11.4          | *               | 4.4        |
|                      | 30                    | 2.8           | *               | 10.1          | *               | 12.5       |
|                      | 45                    | 0.2           | *               | 6.0           | *               | 8.5        |
| Citronella           | 15                    | 3.0           | 2.22            | 11.5          | 8.44            | 0.8        |
|                      | 30                    | 0.8           | -0.90           | 8.9           | 6.64            | 0.6        |
|                      | 45                    | -0.4          | -2.25           | 5.1           | 4.11            | 0.2        |
|                      | 60                    | -0.6          | -2.07           | 2.5           | 2.06            | 3.1        |
| Clove                | 15                    | 1.8           | 0.54            | 11.2          | 7.99            | 0.01       |
|                      | 30                    | -0.2          | -2.02           | 5.7           | 4.54            | 0.1        |
|                      | 45                    | -0.5          | -1.83           | 2.3           | 1.89            | 1.7        |
|                      | 60                    | -0.8          | -2.82           | 1.4           | 1.14            | 0.2        |

Chi-square value significant at P<0.05 level. \textsuperscript{b}CL: confidence limits LCL: lower confidence limits UCL: upper confidence limits, KD$_{50}$ = Knock-down dose required to kill 50% of the population exposed, KD$_{90}$ = Knock-down dose required to kill 90% of the population exposed. * = Knock-down dose could not be determined.
Table 3b: The KD50 and KD90 of effective essential oils against female adult Cx. quinquefasciatus

| Plant essential oils | Exposure period (min) | 95% CLa (%) | KD50 (%) | KD90 (%) | Chi-square |
|----------------------|-----------------------|-------------|----------|----------|------------|
|                      |                       | LCL        | UCL      | LCL      | UCL        |           |
| Eucalyptus           | 15                    | 3.4        | *        | 11.6     | 4.1        |           |
|                      | 30                    | 1.9        | 0.80     | 2.76     | 10.3       | 6.1        | 0.6       |
|                      | 45                    | 0.3        | -1.54    | 0.83     | 6.1        | 4.88       | 8.92      | 2.2       |
| Lemongrass           | 15                    | 5.3        | *        | 11.2     | *          | *          | 4.0       |
|                      | 30                    | 4.2        | *        | 2.2      | *          | *          | 6.6       |
|                      | 45                    | 1.9        | *        | 7.3      | *          | *          | 4.0       |
| Pine                 | 15                    | 1.5        | 0.23     | 4.52     | 6.50       | 8.95       | 4.73      | 1.3       |
|                      | 30                    | 4.3        | *        | 9.07     | 16.2       | *          | *         | 5.5       |
|                      | 45                    | 1.2        | -2.39    | 2.53     | 14.0       | 8.95       | 4.73      | 1.3       |
| Thyme                | 15                    | 7.5        | *        | 16.5     | *          | *          | 5.1       |
|                      | 30                    | 4.6        | *        | 13.0     | *          | *          | 4.9       |
|                      | 45                    | 1.9        | 0.87     | 2.75     | 10.1       | 7.49       | 17.4      | 2.4       |
| Tulsi                | 15                    | 5.3        | *        | 14.4     | *          | *          | 6.3       |
|                      | 30                    | 4.0        | *        | 12.5     | *          | *          | 4.3       |
|                      | 45                    | 2.3        | *        | 12.9     | *          | *          | 4.3       |
|                      | 60                    | 0.2        | -1.62    | 2.75     | 7.6        | 4.82       | 13.6      | 3.4       |
| Deltamethrin (0.05%) | 15                    | 7.4        | *        | 16.4     | *          | *          | 6.6       |
|                      | 30                    | 4.1        | *        | 11.5     | *          | *          | 5.2       |
|                      | 45                    | 2.5        | *        | 11.9     | *          | *          | 4.5       |
|                      | 60                    | 0.2        | -1.66    | 1.15     | 7.7        | 5.89       | 12.6      | 3.3       |
|                      | 30                    | 0.8        | -0.90    | 1.66     | 8.9        | 6.64       | 15.3      | 0.6       |
|                      | 45                    | -0.4       | -2.25    | 0.38     | 5.1        | 4.11       | 7.28      | 0.2       |
|                      | 60                    | -0.6       | -2.07    | 0.00     | 2.6        | 2.06       | 3.32      | 2.8       |

Chi-square value significant at P<0.05 level, *CL: confidence limits; LCL: lower confidence limits; UCL: upper confidence limits, KD50= Knock-down dose required to kill 50% of the population exposed, KD90= Knock-down dose required to kill 90% of the population exposed. *

DISCUSSION

In the present study 22 essential oils registered knock-down and adulticidal effects. Plenty of literature is available with regard to bio efficacy of volatile oils against vector mosquitoes. These findings are comparable to those of Vartak and Sharma [22] have reported the knock-down effect of terpenoids of volatile oils against A. aegypti adult females.

Prajapati et al. [23] have studied the essential oils viz., Cinnamomum zeylanicum, Cuminum cyminum, Cyperus scariosus, Carcuma longa, Juniperus macropoda, Ocimum basilicum, Rosmarinus officinalis, Nigella sativa, Pimpinella anisum, and Zingiber officinalis for adulticidal activity against three mosquito species; Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. Omolosa et al. [24] have reported the fumigant toxicity of essential oils from 15 species of African plants against Anopheles gambiae in the laboratory. They reported that oils of 6 plant species viz., Tarchonanthus camphoratus, Lippia javanica, Plectranthus mauritoides, Tetradenia riparia, Lippia ukambensis and Conya newil were found to be relatively more toxic, with C. newil and P. mauritoides showing the highest potency.

Rajkumar and Jabanesan [25] reported the knocking down and killing effects of Solanum arietanthum D. Don. leaf extracts against the mosquito Culex quinquefasciatus Say. Jeyabal et al. [26] have reported the adulticidal effect of Pelargonium citrona on Anopheles stephensi, with LC50 and LC90 values as 1.56% and 5.22% respectively. Nathan et al. [27], who reported the adulticidal activity of methanol extract of Dysosylym malabaricum leaves against Anopheles stephensi. Adulticidal activity of five essential oils (Citrus sinensis, Mentha piperita, Carvoryl oil, Citronella oil and citral oil) at different concentrations and time intervals was determined. The Rutaceae oil (C. sinensis) was found as the most toxic against Cx. Quinquefasciatus [28]. A large number of plants are reported to have adulticidal activity [28, 29-30]. Kovendan et al. [31] found the adulticidal activity of methanol extract of Acalypa alinifolia leaves against three mosquito species, Ae. aegypti, An. stephensi and Culex quinquefasciatus. Dua et al. [30] reported the adulticidal activity of essential oil of Valeriana jatamansi root against An. stephensi, An. culicifacies, Ae. aegypti, Ae. albopictus, and Cx. quinquefasciatus, with LD50 and LD90 values were 0.14, 0.16, 0.09, 0.08, 0.17 mg/cm2 and 0.24, 0.34, 0.25, 0.21, 0.28 mg/cm2, respectively. Whereas KDT50 and KDT90 values were 13, 13, 12, 13, 18 min and 24, 25, 21, 20, 42 min against An. stephensi, An. culicifacies, Ae. aegypti, An. albopictus and Cx. quinquefasciatus, respectively, using 0.28 mg/cm2 impregnated papers.

Yang et al. [32] have studied the adulticidal activity of five essential oils against Culex pipiens. They found that the Rutaceae oil obtained from Citrus sinensis was the most effective adulticidal treatment. Other plant species that are reported to possess adulticidal activity includes: Carcuma aromatic against Ae. aegypti [17]. The adulticidal activity of ethanol extract of Aipun graven olens seeds against Aedes aegypti has been reported [33]. Dua et al. [29] reported the adulticidal activity of essential oil of leaves of Lantana camara against Ae. aegypti, Cx. quinquefasciatus, An. culicifacies, An. fluviatilis and An. stephensi, LD50 values were 0.06, 0.05, 0.05, 0.05 and 0.06 mg/cm2 while LD90 values were 0.10, 0.10, 0.09, 0.09 and 0.10 mg/cm2 respectively. Whereas KDT50 values were 20, 18, 15, 12, 14 min and KDT90 values were 35, 28, 25, 18, and 23 min against Ae. aegypti, Cx. quinquefasciatus, An. culicifacies, An. fluviatilis and An. stephensi, respectively on 0.208 mg/cm2 impregnated paper. The target of many insecticides is the nervous system and in particular, acetylcholine esterase (AChE). However little information is available on mode of adulticidal activity of essential oil. Ware [34] (1994) has reported that the only way volatile insecticides can enter the pest’s body is through the respiratory system.

In the present study the volatile oils showed variation in Knock-down and adulticidal activity when tested by impregnated filter paper method. Calamus, camphor, cinnamon, citronella, clove, eucalyptus, lemongrass, pine, thyme and tulu oils recorded 100 per cent Knock-down and adult mortality at 10%/cm2 dose only in WHO.

---

**Int J Pharm Pharm Sci, Vol 9, Issue 5, 156-160**
ACKNOWLEDGEMENT

The present study could be useful in potential to be developed as an eco-friendly insecticide against mosquito. The results of the present study could be useful in developing new insecticidal agents for adult mosquito control with filter paper method. The impregnated paper assay finding may be utilized for the development of botanical based insecticides as alternative to synthetic insecticides for adult mosquito control.

CONFLICT OF INTERESTS

Declared none.

REFERENCES

1. Jang YS, Ahn YJ, Lee HS. Larvicidal activity of Brazilian plants against Aedes aegypti and Culex pipiens (Diptera: Culicidae). Agric Chem Biotechnol 2002;45:131-4.
2. Agrawal VK, Sashidran VK. Lymphatic filariasis in India problems, challenges and new initiatives. Med J Armed Forces India 2006;62:559-62.
3. Githoko AK, Lindsay SW, Confloloneri UE, Patz JA. Climate change and vector-borne diseases: a regional analysis. Bull WHO 2000;78:1136-47.
4. Fox LM. Infectious Diseases Related To Travel. In CDC Health Information for International Travel 2012. Edited by Brunette GW. New York: Oxford University Press; 2011.
5. Peng Z, Yang J, Wang H, Simons FER. Production and characterization of monocalonal antibodies to two new mosquito aedes aegypti salivary proteins. Insect Biochem Mol Biol 1999;29:909-14.
6. Severini C, Romi R, Marinucci M, Raymond M. Mechanisms of insecticides resistance in field population of Culex pipiens from Italy. J Am Mosq Control Assoc 1993;9:164-8.
7. Curtis CF, Lines JD, Bu B, Renze A. Natural and synthetic repellents. In: Appropriate Technology in Vector Control. CF Curtis. Ed. Boca Raton; 1990. p. 75–92.
8. Zaim M, Jambulingam P. Global insecticide use for vector-borne disease control. World Health Organization Pesticide Evaluation Scheme (WHOPE). In: World Health Organization Pesticide Evaluation Scheme (WHOPE): World Health Organization: Geneva; 2004.
9. Brogdon WG, McAllister JC. Insecticide resistance and vector control Emerging Infect Dis 1998;4:605–13.
10. WHO. Expert Committee on Vector Biology and Control Sixteenth report. Geneva; 1999. p. EB108/6.
11. Bowers WS, Sener B, Evans H, Binalg F, Erodogan I. Activity of organochlorine, organophosphate and carbamate insecticides: susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides: diagnostic test. Vol. WHO/VBC/81.807. Geneva; 1981.
12. Duncan BD. Multiple Comparisons Among Means. Biometrics 1955;13:359–64.
13. Finney DJ. In: Probit Analysis. 3rd ed. edition. 32 E 57th St. New York: Cambridge University Press 1971.
14. Abbott WS. A method of computing the effectiveness of an insecticide. 1925. J Am Mosq Control Assoc 1987;3:302-3.
15. Vartak PH, Sharma RN. Vapour toxicity and repellence of some essential oils and terpenoids to adults of Aedes aegypti (L) (Diptera: Culicidae). Indian Med Res 1993;97:12–7.
16. Prajapati VV, Tripathi AK, Aggarwal KK, Khanuja SPS. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. Bioresearch Technol 2005;96:1749–57.
17. Omolo MO, Okinyo D, Ndige IO, Lwandeb W, Hassanali A. Fumigant toxicity of the essential oils of some African plants against Anopheles gambiae sensu stricto. Pharmcy Medicine 2005;12:241–6.
18. World Health Organization. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides: diagnostic test. Vol. WHO/VBC/81.807. Geneva; 1981.
19. How to cite this article

- M Ramar, S Ignacimuthu, P Manonmani, K Murugan. Adulticidal activity of botanical oils by impregnated paper assay against Culex quinquefasciatus say. Int J Pharm Pharm Sci 2017;9(5):156-160.