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Larvicidal Efficacy of Fifteen Plant Essential Oils against Culex pipiens L. Mosquitoes in Egypt

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Introduction

Mosquito-borne diseases represent a significant threat to human/animal health and as an impediment to socio-economic development [1, 2] due to their wide geographical distribution, the rapid development of vector resistance, the spread of drug resistance to pathogens they transmit and the unavailability of effective vaccines against many mosquito-borne-diseases [3]. The situation is further complicated due to global warming, climate change and the worldwide distribution of disease vectors including Culex pipiens L. The widely distributed house mosquito, Cx. pipiens was responsible for 1977, 1978, 1993 Egyptian epidemics of Rift Valley fever [4] and the...
widespread transmission of Bancroft filariasis in the Nile Delta [5].

Using repellents and larvicides to control mosquito larval stages at their aquatic habitats, rather than adulticides which only temporarily reduce the adult populations, are the most effective approaches for reducing mosquito bites nosiness and their associated mosquito-borne diseases. Synthetic insecticides led to insecticide resistance, environmental pollution, impact non-target organisms and health hazards to humans [6]. Consequently, new strategies and technologies for applying natural insecticides are needed to combat the increasing mosquito resistance to chemical insecticides [6, 7] and reduce the detrimental effects of chemicals on the environment and on human health.

Essential Oils (EOs) can be used as alternatives to synthetic insecticides [8-13]. The EOs are safer phytochemicals due to a long history of use for human consumption, fragrances, and medicines [14, 15, 16, 17, 18, 19]. Many reports documented the effective use of plant extracts against mosquito larvae and their safety in non-target organisms [21-23]. Previous studies have demonstrated the importance of EOs as alternatives to synthetic insecticides [24, 25].

This study evaluated the use of mosquitocidal activities of 15 plant essential oils of which two were novel. Their lethal concentrations and relative effectiveness against the larvae of Cx. pipiens were determined.

### Material and Methods

#### Plant Essential Oils

Fifteen plant EOs were used in this study, all of them were purchased from EL Captain company for extracting natural oils, plants, and cosmetics “Cap Pharm,” El Obour, Cairo, Egypt, except for caster and fenugreek oils, purchased from Harraz store for Food Industry and Natural products, Cairo, Egypt (Table 1).

#### Mosquitoes

The immature stages of Cx. pipiens were obtained from a colony in the Department of Entomology, Faculty of Science, Banha University, Egypt, collected initially from old natural waterways in the Sheiblnja village, Banha-Egypt, and maintained at 26.5±1°C, 70-80% relative humidity, and 16/8 hours light/dark photoperiods.

#### Larvicidal Efficacy

The larvicidal efficacies of fifteen plant EOs were evaluated against the early fourth instar larvae of Cx. pipiens [26]. The EOs were diluted in a solvent consisting of dechlorinated water and 5% Tween 20 as an emulsifier [13]. Twenty early fourth larval instars of Cx. pipiens were exposed to each EO at different concentrations (125, 250, 500, 1,000, and 2,000 ppm). The testing and the control group, treated with the solvent only, were replicated three times. Mortalities were monitored 24 hours post-treatment.

### TABLE 1. Plant essential oils used against fourth instar larvae of Culex pipiens.

<table>
<thead>
<tr>
<th>Essential Oil Species</th>
<th>Family</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Allium sativum</td>
<td>Amaryllidaceae</td>
<td>Garlic</td>
</tr>
<tr>
<td>Brassica compestris</td>
<td>Brassicaceae</td>
<td>brown mustard</td>
</tr>
<tr>
<td>*Carum petroselinum</td>
<td>Apiaceae</td>
<td>parsley</td>
</tr>
<tr>
<td>*Cinnamomum verum</td>
<td>Lauraceae</td>
<td>Ceylon cinnamon tree</td>
</tr>
<tr>
<td>*Cocos nucifera</td>
<td>Arecales</td>
<td>Coconut</td>
</tr>
<tr>
<td>*Jasminum sambac</td>
<td>Oleaceae</td>
<td>Arabian jasmine</td>
</tr>
<tr>
<td>*Lavandula angustifolia</td>
<td>Lamiaceae</td>
<td>lavender</td>
</tr>
<tr>
<td>Matricaria chamomilla</td>
<td>Asteraceae</td>
<td>chamomile</td>
</tr>
<tr>
<td>*Pimpinella anisum</td>
<td>Apiaceae</td>
<td>Anise</td>
</tr>
<tr>
<td>*Ricinus communis</td>
<td>Euphorbiaceae</td>
<td>Castor</td>
</tr>
<tr>
<td>Rosmarinus officinalis</td>
<td>Lamiaceae</td>
<td>Rosemary</td>
</tr>
<tr>
<td>*Simmondsia chinensis</td>
<td>Simmondsiaceae</td>
<td>Jojoba</td>
</tr>
<tr>
<td>Trigonella foenum</td>
<td>Fabaceae</td>
<td>Fenugreek</td>
</tr>
<tr>
<td>*Vitis vinifera</td>
<td>Vitaceae</td>
<td>Grape</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>Zingiberaceae</td>
<td>Garden ginger</td>
</tr>
</tbody>
</table>

* plant oils with novel use against Cx. pipiens.
Statistical analysis

Data analysis were done via the one-way analysis of variance (ANOVA), Duncan’s multiple range tests, as well as the Probit analysis to calculate the lethal concentrations (LC) using the computer program PASW Statistics 2009 (SPSS version 22). The efficacies, as well as relative efficacies of EOs were calculated.

Results

The susceptibility of fourth instar larvae of Cx. pipiens to 15 EOs were evaluated. EOs were classified into three groups according to their mortality % (> 90% (eight oils), 90% (four oils), and < 90% (three oils), 24 h post treatment with 2000 ppm. The highly effective group (H group) provided 91-100% mortality and included Ricinus communis, Pimpinella anisum, Matricaria chamomelum, Vitis vinifera, Allium sativum, Jasminum sambac, Cinnamomum verum, and Rosmarinus officinalis (100, 98.33, 98.33, 98.33, 91.67, 93.33, 93.35, and 96.67, respectively).

The moderately effective group (M group) resulted in 90% mortality and contained Trigonella foenum-graecum, Simmondsia chinensis, Brassica kompskri, and Carum petroselinum. The least effective group (L group) resulted in less than 90% mortality and included Cocos nucifera, Zingiber officinalis, and Lavandula angustifolia. R. communis was the most effective oil, whereas C. nucifera was the least effective one (Fig. 1).

The LC$_{50}$ of the H group ranged from 454.48 ppm for R. communis to 754.30 ppm for C. verum, and their values LC$_{99}$ ranged from 1,284.51 to 2,136.68 ppm. The LC$_{50}$ values of M group varied from 823.84 ppm for C. petroselinum and 1,120.91 ppm for S. chinensis and their LC$_{99}$ values were 2,463.95 to 3,021.93 ppm. Regarding relative efficacy, R. communis, M. chamomilla, V. vinifera, and R. officinalis killed larvae 3.8, 3.7, 3.1, and 2.8 times more effectively than C. nucifera as the reference EO (Table 2).

Bars denote standard error.

Fig. 1. Mortality percentage of fourth instar larvae of Culex pipiens post-treatment with five concentrations of plant essential oils.
### Table 2. Susceptibility tests of fourth instar larvae of *Culex pipiens* to plant essential oils (24 hours post-treatment)

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>LC50 (Upper-Lower)</th>
<th>LC50 (Upper-Lower)</th>
<th>LC50 (Upper-Lower)</th>
<th>LC50 (Upper-Lower)</th>
<th>χ²</th>
<th>RE LC50</th>
<th>RE LC50</th>
<th>RE LC50</th>
<th>RE LC50</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Allium sativum</em></td>
<td>1480.14 (930.57-6454.51)</td>
<td>1480.14 (930.57-6454.51)</td>
<td>1715.40 (1078.94-7843.47)</td>
<td>2156.69 (1342.31-10463.88)</td>
<td>36.207*</td>
<td>1.4</td>
<td>2.4</td>
<td>2.4</td>
<td>2.3</td>
</tr>
<tr>
<td><em>Brassica compestris</em></td>
<td>852.45 (1258.62-1000.21)</td>
<td>1725.05 (1432.03-3520.92)</td>
<td>1983.48 (1748.90-4606.08)</td>
<td>2463.95 (1748.06-4840.91)</td>
<td>16.409*</td>
<td>2.4</td>
<td>2.1</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td><em>Carum petrosum</em></td>
<td>823.84 (1245.96-3218.68)</td>
<td>1515.82 (1421.64-3779.68)</td>
<td>1731.72 (1748.06-4840.91)</td>
<td>2136.68 (1748.06-4840.91)</td>
<td>16.608*</td>
<td>2.5</td>
<td>2.1</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td><em>Cinnamomum verum</em></td>
<td>754.30 (1073.08-3072.33)</td>
<td>1515.82 (1421.64-3779.68)</td>
<td>1731.72 (1748.06-4840.91)</td>
<td>2136.68 (1748.06-4840.91)</td>
<td>20.434*</td>
<td>2.7</td>
<td>2.4</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td><em>Cocos nucifera</em></td>
<td>508.30-1350.60</td>
<td>3596.73 (1808.50-3601.86)</td>
<td>4041.43 (1488.37-4603.83)</td>
<td>4875.60 (1488.37-4603.83)</td>
<td>8.523</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Jasminum sambac</em></td>
<td>2028.06 (2988.83-4648.26)</td>
<td>2988.83 (2988.83-4648.26)</td>
<td>3341.06-5259.22 (3341.06-5259.22)</td>
<td>3999.45-6407.61 (3999.45-6407.61)</td>
<td>30.599*</td>
<td>3.0</td>
<td>2.5</td>
<td>2.4</td>
<td>2.3</td>
</tr>
<tr>
<td><em>Lavandula angustifolia</em></td>
<td>675.10 (948.12-4586.98)</td>
<td>1450.21 (1089.52-5496.47)</td>
<td>1669.99 (1342.67-7214.62)</td>
<td>2082.19 (1342.67-7214.62)</td>
<td>19.744*</td>
<td>2.1</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td><em>Maticaria chamomilla</em></td>
<td>454.48 (693.75-1784.55)</td>
<td>941.43 (1563.95-4716.90)</td>
<td>1076.03 (1912.37-6044.82)</td>
<td>1328.31 (1912.37-6044.82)</td>
<td>11.705*</td>
<td>4.3</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
</tr>
<tr>
<td><em>Pimpinella anisum</em></td>
<td>309.78-751.99</td>
<td>1340.14 (785.38-2102.82)</td>
<td>1520.01 (952.39-2704.72)</td>
<td>1875.43 (952.39-2704.72)</td>
<td>13.823*</td>
<td>4.3</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
</tr>
<tr>
<td><em>Ricinus communis</em></td>
<td>474.43-1133.91</td>
<td>911.73 (1103.20-2850.39)</td>
<td>1041.36 (1350.08-3653.45)</td>
<td>1284.31 (1350.08-3653.45)</td>
<td>15.579</td>
<td>2.9</td>
<td>2.7</td>
<td>2.7</td>
<td>2.6</td>
</tr>
<tr>
<td><em>Rosmarinus officinalis</em></td>
<td>360.76 (881.59-1556.49)</td>
<td>8442.85 (773.88-1820.38)</td>
<td>1413.18 (941.99-2320.99)</td>
<td>1769.18 (941.99-2320.99)</td>
<td>39.232*</td>
<td>4.5</td>
<td>3.9</td>
<td>3.9</td>
<td>3.8</td>
</tr>
<tr>
<td><em>Simmondsia chinensis</em></td>
<td>294.38-866.53</td>
<td>1120.91 (759.34-8442.39)</td>
<td>2213.11 (875.79-10308.11)</td>
<td>3021.93 (1081.53-13819.81)</td>
<td>17.831*</td>
<td>3.6</td>
<td>0.4</td>
<td>2.9</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Trigonella foenum</em></td>
<td>764.28-2134.96</td>
<td>759.34-8442.39 (875.79-10308.11)</td>
<td>2213.11 (875.79-10308.11)</td>
<td>3021.93 (1081.53-13819.81)</td>
<td>17.831*</td>
<td>3.6</td>
<td>0.4</td>
<td>2.9</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Vitis vinifera</em></td>
<td>546.91 (1589.51-4235.14)</td>
<td>1724.60 (1631.33-4111.95)</td>
<td>1978.26 (2121.52-6154.03)</td>
<td>2494.07 (2121.52-6154.03)</td>
<td>19.326*</td>
<td>4.2</td>
<td>2.1</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td><em>Zingiber officinale</em></td>
<td>1129.91 (1223.06-3445.55)</td>
<td>1103.91 (1396.25-4051.80)</td>
<td>1261.81 (1711.58-5199.84)</td>
<td>1538.00 (1711.58-5199.84)</td>
<td>50.144*</td>
<td>3.7</td>
<td>3.3</td>
<td>3.2</td>
<td>3.1</td>
</tr>
</tbody>
</table>

*χ²: Chi-square values with asterisk are significant P>0.05; RE: relative efficiency according to that of *Cocos nucifera* as a reference oil*
Discussion

Because environmental safety is of paramount importance, an insecticide needs to be highly effective and eco-friendly at the same time. Botanicals, including EOs have a long history of successful application in ethnoveterinary medicine and continue to be used as a suitable insecticide [27].

This study demonstrated the lethal potentials of 15 plant EOs, including two novel ones, C. nucifera and J. sambac, against fourth instar larvae of Cx. pipiens.

Our findings indicated that the most effective oils post treatment with 2000 ppm were R. communis (100 MO%); M. chamomilla and V. vinifera (98.33%); and R. officinalis (96.67 MO%) and their LC50 values were 454.48, 466.64, 546.91, and 560.76 ppm, respectively. Analogous to our finding, R. communis has demonstrated high effectiveness against Cx. pipiens, Aedes caspius, Culiseta longiareolata, and Anopheles maculipennis in rural areas of Mohammedia, a coastal city on the Atlantic Moroccan [28] as well as Culex pipiens. larvae in collected in the District of Monte Santo, Campina Grande, State of Paraiba, Brazil [29, 30]. Also, similarly to our findings, the larvicidal efficacy of M. chamomillawas confirmed against the growth and development of Cx. pipiens larvae in Riyadh, KSA [31].

Our data indicated that V. vinifera was one of the highly effective EOs, like the effect that was recorded against Cx. pipiens larvae in Egypt by [32]. Similar effects of R. officinalis were reported for its different chemical extracts against Cx. pipiens larvae [33]. In addition, R. officinalis oil was demonstrated highly toxic to the first instar larvae of Ae. aegypti but was not toxic at the highest concentration tested against older larval instars [34]. P. anisum was one of the H group EOs in this study and similarly it was toxic against the larvae of Cx. pipiens in Greece [35], and larvae and adults of Cx. quinquefasciatus in relatively low concentrations [36]. A. sativum was also highly effective in this study. Likewise, its aqueous extract effectively controlled Anopheles and Culex mosquito larvae [37] and the larvae of related species, An. cepa, Cx. pipiens, and Musca domestica in Egypt [38]. C. verum showed high larvicidal activity against Cx. pipiens, p.pipiens in this study, and likewise, cinnamon oil was a good larvicide against Cx. pipiens pullens and as repellent and fumigant against female Cx. pipiens [39]. In this study, B. compestris, C. petroselinum, S. chinensis, and T. foenum-graecum provided a 90% larvicidal effect (M group). Similarly, those oils have shown larvicidal effects and alteration of some biological aspectsdevelopmental periods, pupation rates, and adult emergence of Cx. pipiens [40]. C. petroselinum was moderately effective against Cx. pipiens larvae in this study, but not effective in another study conducted in Iraq [41]. S. chinensis (2000 ppm) provided 90% mortality in this study and some of its fractions have also been shown to be effective against Cx. pipiens larvae [42]. However, S. chinensis was less effective against Cx. quinquefasciatus as100 and 26.7% mortalities were reached after treatment with higher concentrations, 12,000 and 4,000 ppm, respectively [43].

Z. officinale provided a low toxic larvicidal effect against Cx. pipiens larvae (78.33%) in this study. In contrast, all larval stages of An. culicoides showed full susceptibility to Z. officinale oil at 25 ppm [44]. On the other hand, high concentrations of Z. officinale powder showed a greater larvicidal effect against third larval instars of Cx. pipiens [45]. Our data indicated that C. nucifera (45% MO) was the least effective EO. Likewise, a relatively high concentration of the coconut fatty acids was required to suppress the growth of the late third instar larvae of Ae. aegypti mosquitoes [46]. Also, L. angustifolia was found to be less effective EO (85.00% MO), while it was found to be highly effective against Cx. pipiens larvae in Algeria and Morocco [47, 48]. Resins of Commiphora molmol, Aervafrica heterophylla, Eucalyptus camaldulensis, Pistacia lentiscus, and Boswellia sacra controlled the fourth larval instars of Cx. p p i e n s in Egypt [7]. Some of the applied EOs in current study had insecticidal effect against some other dipteran flies, T. foenum and B. compestris [49] and M. chamomilla, P. anisum, R. officinalis [9] were effective against Lucilia sericata, A. sativum [11] and L. angustifolia, Cinnamomum camphora, and Allium cepa [10] induced larvicidal effect against the oestrud fly, Cephalopina titillator (Clark). M. chamomilla, and R. officinalis effectively controlled the buffalo lice, Haematopinus tuberculatus, and repelled flies infesting water buffaloes in Egypt [8]. Moreover, C. zeylanicum, and L. angustifolia provided ovicidal, larvicidal, adulticidal, repellent and oviposition deterrent effect against M. domestica and L. sericata [12, 50, 51].

This study demonstrated the lethal potentials of 15 plant EOs, including two novel ones, *C. nucifera* and *J. sambac*. It is recommended to use *R. communis, M. chamomilla*, and *V. vinifera* for field application. Generally, such eco-friendly low-cost EOs can be used in safe phytochemical insecticides to manage mosquito vectors in local, regional, and rural communities that have fewer other control options. Further investigations are needed for applicable formulations for the recommended EOs to enhance efficacy, persistence, and guarantee sufficient spreading crosswise water surface in mosquito larval control.

**Author Contributions:** H. F.: helped designing and choosing the plants, doing the experiment and writing work. D. S.: helped with writing the manuscript and editing the manuscript; M. D.: helped in editing the manuscript; and M. M. B.: helped to do laboratory work.

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**Conflict of interests**

The authors declare no conflict of interest.

**Ethics approval, Consent, Data, Material and/or Code availability**

Not abdicable

**Competing Interests**

The authors have no competing interests to declare that are relevant to the content of this article.

**References**


فعالية مبيدات مميتات اليرقات لخمسة عشر زيوت نباتية ضد البعوض في مصر

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قسم الطفيليات، كلية الطب البيطرى جامعة بنها. طوخ
قسم الحشرات كلية العلوم، جامعة عين شمس - القاهرة
قسم الأمراض المعدية كلية الطب البيطرى، جامعة بنها - طوخ
منطقة دلتا لمكافحة البعوض والنواقل، فساليا، كاليفورنيا، الولايات المتحدة
قسم علم الحشرات، كلية العلوم، جامعة بنها - بنها

ال البعوض Culex pipiens هي ناقلات مهمة لنقل حمى الوادي المتصدع وداء الفيلارياء الليمفاوية في مصر. تعتبر الزيوت الأساسية النباتية (EOs) واحدة من أكثر مبيدات اليرقات فعالية. قمت هذه الدراسة فعالية EOs لمبيدات اليرقات لـ Cx. pipiens النائم منها جنديان ضد البعوض في العمر الرابع من تطبيق EOs على البعوض لمدة 24 ساعة، ثم تضمنت الدراسة تطبيق EOs لمدة 2000 ساعة لمدة MOs (من 125 إلى 1000 و2000 جزء في المليون) لكل زيت. تم تصنيف الزيوت الأسلوبية في ثلاث مجموعات تبعاً لفعالية الدراسة. قدمت المجموعة عالية الفعالية تحتوي على Pimpinella anisum وRicinus communis، وأظهرت أن تركيزها القاتل (LC50) مخلوطة و100 إلى 91٪ من LC50. تراوحت ومتراوحة تركيزاتها القاتل (MO) من 100 إلى 754.30٪ من LC50. أمضت المجموعة القاتلة بشكل LC50 من LC50 ازدادت EOs لمبيدات اليرقات في المليون Carum Petroselinum وBrassica compestris وLavandula angustifolia وZingiber officinale وCocos nucifera وR. communis وC. pipiens. تم استخدام هذه الدراسة كمبيدات بشرى صديقة للبيئة ومتعددة الأفكارية بشكل LC50 