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

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THE mosquitoes *Culex pipiens* are important vectors for transmitting Rift Valley fever and lymphatic filariasis in Egypt. Plant essential oils (EOs) are one of the most promising larvicides. This study assessed the larvicidal efficacy of 15 EOs of which two were new against fourth larval instars of *Cx. pipiens*. Five concentrations (125, 250, 500, 1,000, and 2,000 ppm) were used for each oil. Mortalities (MOs) were monitored 24 hours post-treatment. After treatment with 2000 ppm for 24 hours, EOs were classified into three groups**.** The highly effective group provided eight EOs ranging from 91 to 100% MO including*Ricinus communis*, *Pimpinella anisum*, *Matricaria chamomilla*, *Vitis vinifera*, *Allium sativum*, *Jasminum sambac*, *Cinnamomum verum*, and *Rosmarinus officinalis*. Their lethal concentrations (LC_{50}) ranged from 454.48 ppm (*R. communis*) to 754.30 ppm(*C. verum*). The moderately effective group resulted in 90% MO by *Trigonella foenum-graecum*, *Simmondsia chinensis*, *Brassica compestris*, and *Carum Petroselinum*. The LC_{50} values varied from 823.84 ppm (*C. petroselinum*)to 1,120.91 ppm (*S. chinensis*). The least effective group provided less than 90% mortality and included *Cocos nucifera*, *Zingiber officinale*, and *Lavandula angustifolia*.*C. nucifera* and*J. sambac* were novelty used against *Cx. pipiens*. *R. communis,* and *M. chamomilla* were recommended for field application as eco-friendly larvicides.

Keywords: Mosquito control, Larvicides, Essential oils, Lethal concentrations, *Culex pipiens.*

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

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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,- 20].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 form 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.*

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

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

Statistical analysis

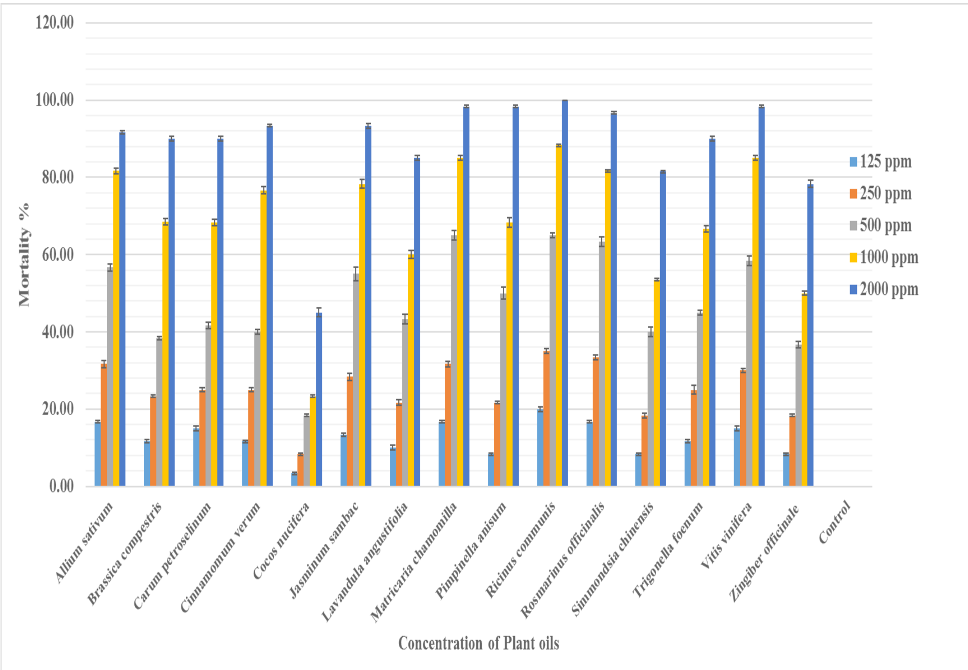
Data analysis were done via the one-way analysis of variance (ANOVA), Duncan's multiple range tests, as well as the Probit analysis to calculate the lethal concentrations (LC) using the computer program PASW Statistics 2009 (SPSS version 22). The efficacies, as well as relative efficacies of EOs were 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 chamaemelum*, *Vitis vinifera*, *Allium sativum*, *Jasminum sambac*, *Cinnamomum velum*, 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 compestris*, and *Carum petroselinum*. The least effective group (L group) resulted in less than 90% mortality and included *Cocos nucifera*, *Zingiber officinale*, 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)

	LC_{50} (Upper-Lower)	LC_{90} (Upper-Lower)	LC_{95} (Upper-Lower)	LC_{99} (Upper-Lower)	$\chi 2$	RE LC_{50}	RE LC_{90}	RE LC_{95}	RE LC_{99}
Allium sativum	1480.14 (930.57-6454.51)	1480.14 (930.57-6454.51)	1715.40 (1078.94-7843.47)	2156.69 (1342.31-10463.88)	36.207*	1.4	2.4	2.4	2.3
Brassica compestris	852.45 (547.89- 1362.42)	1725.09 (1258.62-3100.21	1972.47 (1432.03- 3620.92)	2436.51 (1748.90-4606.08)	16.409*	2.4	2.1	2.0	2.0
Carum petroselinum	823.84 (508.30- 1350.60)	1727.35 (1245.96-3218.68	1983.48 (1423.64-3779.68)	2463.95 (1748.06- 4840.91)	16.808*	2.5	2.1	2.0	2.0
Cinnamomum verum	754.30 (447.71- 1309.73)	1515.83 (1073.08-3072.33	1731.72 (1220.50-3601.86)	2136.68 (1488.37-4603.83)	20.434*	2.7	2.4	2.3	2.3
Cocos nucifera	2028.06 (1724.87-2514.56)	3596.73 (2988.83-4648.26)	4041.43 (3341.06-5259.22)	4875.60 (3999.45-6407.61)	8.523	1.0	1.0	1.0	1.0
Jasminum sambac	675.10 (261.48-1566.61)	1450.25 (948.12-4586.98)	1669.99 (1089.52- 5496.47)	2082.19 (1342.67- 7214.62)	30.998*	3.0	2.5	2.4	2.3
Lavandula angustifolia	947.04 (571.54- 1663.38)	1946.32 (1372.85-4014.36)	2229.60 (1563.95-4716.90)	2760.98 (1912.37-6044.82)	19.744*	2.1	1.8	1.8	1.8
Matricaria chamomilla	466.64 (300.78- 731.59)	941.43 (693.75-1784.55)	1076.03 (785.38-2102.82)	1328.51 (952.39-2704.72)	17.075*	4.3	3.8	3.8	3.
Pimpinella anisum	705.63 (474.43- 1133.91)	1340.14 (981.081-2479.44)	1520.01 (1105.20-2880.39)	1857.43 (1332.08- 3638.45)	15.823*	2.9	2.7	2.7	2.6
Ricinus communis	454.48 (294.38-686.53)	911.739 (681.59- 1556.49)	1041.36 (773.88-1820.58)	1284.51 (941.99-2320.99)	13.579	4.5	3.9	3.9	3.8
Rosmarinus officinalis	560.76 (76.48- 2134.96)	8442.89 (759.34-8442.89	1415.18 (875.79-10308.11)	1769.18 (1081.53-13819.81)	39.232*	3.6	0.4	2.9	2.8
Simmondsia chinensis	1120.91 (751.26-1833.73)	2213.11 (1589.51-4235.14	2359.59 (1631.33-4511.95)	3021.93 (2121.52-6154.03)	17.831*	1.8	1.6	1.7	1.6
Trigonella foenum	829.84 (491.58-1426.67)	1724.60 (1223.06-3445.35)	1978.26 (1396.25-4051.80)	2454.07 (1711.58-5198.94)	19.326*	4.2	2.1	2.0	2.0
Vitis vinifera	546.91 (333.54-921.39)	1103.91 (665.586-1636.79)	1261.81 (761.802-1896.191)	1558.00 (929.46-2882.82)	50.144*	3.7	3.3	3.2	3.1
Zingiber officinale	1129.91 (755.26-1883.71)	2243.81 (1616.55-4295.00)	2559.59 (1831.33-5007.95)	3151.93 (2225.52-6354.03)	15.931*	1.8	1.6	1.6	1.5

.$\chi 2$: 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 successfulapplicationin 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 were454.48, 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 *Ae. aegypti* larvae in collected in the District of Monte Santo, Campina Grande, State of Paraíba, Brazil[29, 30]. Also, similarly to our findings, the larvicidal efficacy of *M. chamomilla*was 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. pipiens* in this study, andlikewise, cinnamon oil was a good larvicide against *Cx. pipiens pallens* 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. coluzzii* 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*, *Araucaria heterophylla*, *Eucalyptus camaldulensis*, *Pistacia lentiscus*, and *Boswellia sacra* controlled the fourth larval instars of *Cx. pipiens*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 agianst *Lucilia sericata*.*A. sativum* [11] and *L. angustifolia*, *Cinnamomum camphora,* and *Allium cepa*[10] inducedlarvicidal effect against the oestrid fly, *Cephalopina titillator* (Clark). *M. chamomilla,* and *R. officinalis* effectively controlled the buffalo lice, *Haematopinus tuberculatus,* and repelled fliesinfesting 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.

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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.

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فعالية مبيدات مميتاتاليرقات لخمسة عشر زيوت نباتية ضد بعوضCulex pipiens L في مصر.

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البعوض Culex pipiens هي ناقلات مهمة لنقل حمى الوادي المتصدع وداءالفيلارياالليمفاوية في مصر. تعتبر الزيوت الأساسية النباتية (EOs) واحدة من أكثر مبيدات اليرقات الواعدة. قيمت هذه الدراسة فعالية مبيدات اليرقات لـ ١٥ EOs ، اثنان منها جديدان ضد اليرقات فى العمر الرابع من Cx. pipiens. تم استخدام خمسة تركيزات (١٢٥ و ٢٥٠ و ٥٠٠ و ١٠٠٠ و ٢٠٠٠ جزء في المليون) لكل زيت. تم رصد الوفيات (MOs) بعد ٢٤ ساعة من المعالجة. بعد العلاج بـ ٢٠٠٠ جزء في المليون لمدة ٢٤ ساعة ، تم تصنيف الزيوتالنباتيةالأساسية إلى ثلاث مجموعات تبعالفاعليتها. قدمت المجموعة عالية الفعالية تحتوى على ثمانية زيوتنباتية تتراوح من ٩١ إلى ١٠٠٪ MOوهم:Ricinus communisو Pimpinella anisum و Matricaria chamomilla و

Vitis vinifera و Allium sativum و Jasminum sambac و Cinnamomum verum و Rosmarinus officinalis. تراوحت تركيزاتها القاتلة (LC٥٠) من ٤٥٤,٤٨ جزء في المليون

(R communis) إلى ٧٥٤,٣٠ جزء في المليون (C. verum). أسفرت المجموعة الفعالة بشكل معتدل عن ٩٠ ٪ MO بواسطة Trigonella foenum-graecum و Simmondsia chinensis و Brassica compestris و Carum Petroselinum. تراوحت قيم LC٥٠ من ٨٢٣,٨٤ جزء في المليون (PetroselinumC) إلى ١،١٢٠,٩١ جزء في المليون (S. chinensis). قدمت المجموعة الأقل فعالية معدل وفيات أقل من ٩٠٪ وشملت Cocos nucifera و Zingiber officinale و Lavandula angustifolia. تم استخدام C. nucifera و J. sambac حداثة ضد Cx. pipiens. لذلكتوصىالدراسة بـ R. communis و M. chamomilla للتطبيق الميداني كمبيدات يرقات صديقة للبيئة.