



RESEARCH ARTICLE

Comparative Evaluation of Five Oil-Resin Plant Extracts against The Mosquito Larvae, *Culex pipiens* Say (Diptera: Culicidae)

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ABSTRACT

The use of eco-friendly insecticides has recently received great public interest. This study evaluated the larvicidal activity of oil-resins of *Commiphora molmol*, *Araucaria heterophylla*, *Eucalyptus camaldulensis*, *Pistacia lentiscus*, and *Boswellia sacra* against 4th larval instar of *Culex pipiens*. The highest larval mortalities were observed by 1500 ppm acetone extracts of *C. molmol* (83.3% and 100% and LC₅₀= 623.52 and 300.63 ppm) and *A. heterophylla* (75% and 95% and LC₅₀= 826.03 and 384.71 ppm) 24 and 48 h PT, respectively. On the other hand, the aqueous extract of *A. heterophylla* was highly effective, LC₅₀= 2819.85 ppm and 1652.50 ppm, followed by that of *C. molmol*, LC₅₀= 3178.22 and 2322.53 ppm, 24 and 48 h PT, respectively. *P. lentiscus* was the least effective material. The RE values of *C. molmol*, *A. heterophylla*, *E. camaldulensis*, and *B. sacra*, acetone extracts were 3.8, 2.9, 2.2, and 1.5 times, respectively, more toxic than that of *P. lentiscus*. While RE of aqueous extracts were 2.4, 2.7, 1.4, and 2.0 times more toxic than that of *P. lentiscus*, respectively, after 24 h PT. GC-MS analysis indicated the presence of 4,4'-Dimethyl-2,2'-dimethylenebicyclohexyl-3,3'-diene (14.62%) and Copaene (13.64%) as the most dominant constituents in *C. molmol* and *A. heterophylla*, respectively. For the first time, according to our knowledge, this work compared the efficacy of oil-resin plants and evaluated *A. heterophylla*, *E. camaldulensis*, and *B. sacra* as larvicides against *Culex pipiens*. It is recommended to use the acetone extracts of *C. molmol* and *A. heterophylla* for controlling mosquitoes.

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INTRODUCTION

Mosquitoes are vectors of dangerous pathogens spreading epidemics to millions of people and animals around the world (Abbas *et al.*, 2014, 2018; Shaukat *et al.*, 2019). *Culex pipiens* is the main vector of filariasis (Abdel-Shafi *et al.*, 2016) and viral epidemics (Meegan *et al.*, 1980; Selim *et al.*, 2019).

Mosquito control is mainly focused on the use of repellents (Khater *et al.*, 2019) and synthetic chemicals insecticides (Baz, 2013) but the emergence of insect resistance and environmental pollution have resulted from the misuse and overuse of pesticides. Therefore, developing alternative environmentally friendly strategies (Karthi *et al.*, 2020) is an imperative need.

Botanicals are a good parasiticide alternative (Fayaz *et al.*, 2019; Khater *et al.*, 2020; Zaman *et al.*, 2020) because

they are eco-friendly, cost-effective, and biodegradable (Khater 2012) and considered useful larvicides, repellents, and deterrents (Murugan *et al.*, 2015; Roni *et al.*, 2015; Khater and Geden 2018, 2019; Salman *et al.*, 2020). Oil resin plants contain secondary metabolites (terpenoids and phenols) secreted and stored under internal pressure in plant specialized parts located on the surface of pine trees (conifers) or internally, some of which have clear toxicity to insects and microbes (Langenheim, 2003).

Conifers contain families producing resinous terpenoids, only members of Pinaceae and Araucariaceae are the most widespread and famous species among the conifer trees (Langenheim, 2003). Some plant resins have a wide spectrum of biological activity against insects (Khater and Shalaby, 2008; Hoda *et al.* 2016; Baranitharan *et al.*, 2017; Muturi *et al.*, 2020), but there is a lack of information about their efficacy against *Cx. pipiens*.

Consequently, the study aimed to evaluate the larvicidal effect of locally available and affordable resinous plants against *Cx. pipiens* and revealing the chemical analyses of the promising ones.

MATERIALS AND METHODS

Mosquitoes: *Cx. pipiens* larvae were obtained from the Medical and Molecular Entomology Section, Faculty of Science, Benha University, Egypt, and reared according to that of Baz (2013) for six generations at $27\pm 2^\circ\text{C}$ and 75-80% Relative humidity (RH) under a photoperiod of 14:10 h (light/dark) in the insectary.

Plants materials: The resin of Myrrh gum, *Commiphora molmol* Engl. (Burseraceae), frankincense gum, *Boswellia sacra* Flueck. (Burseraceae), and a mastic tree, *Pistacia lentiscus* (Anacardiaceae) were purchased from an herbalist. The resin secreted from the stems of the river red gum, *Eucalyptus camaldulensis* Dehnh. (Myrtaeae) and pine resin, *Araucaria heterophylla* Salisb. (Araucariaceae) trees obtained from the garden of the Faculty of Agriculture, Benha University, Egypt. Plants were identified at Flora and Phytotaxonomy section, Agricultural Research Center, Giza, Egypt.

Plant extracts: The aqueous extract was prepared as follows: 10 g of each plant oil-resin was grounded; mixed with 100 ml of distilled water (10%), and magnetically mixed for 60 minutes at 35°C at 300 rpm in a shaker incubator before being filtered using Whatman filter paper.

Similarly, the acetone extract was prepared with the exception that the solution was left at room temperature $27\pm 2^\circ\text{C}$ for 48 h in the dark for drying of the raw materials. Finally, the stock extract solutions after evaporation resulted in 3.4-5.6 g/ml.

Bioassays: Different concentrations of acetone and aqueous oil-resin extracts (75, 150, 300, 600, 1200, and 1500 ppm) were prepared and tested against the early 4th larval instar of *Cx. pipiens* (WHO, 1981).

Twenty mosquito larvae were placed in a glass beaker (250 ml) containing de-chlorinated water for each of the experimental concentrations. The experiments were replicated three times with an untreated control group. Mortalities were recorded 24 h post-treatment (PT).

Analysis of plant oil-resin extracts by GC-MS: Extract solutions from promising plants, *C. molmol*, and *A. heterophylla* were analyzed by GC-MS along with Agilent mass spectrometry (Mostafa and Essawy, 2019). The components were determined by comparing their retention durations and mass spectra with those in the mass spectrum database WILEY 09 and NIST 11.

Data analyses: The biological data were subjected to one-way analysis of variance (ANOVA), Duncan's multiple range tests, and Probit analysis using the computer program PASW Statistics 2009 (SPSS version 22). The relative efficacies (RE) were calculated (Khater and Geden 2018).

RE LC = LC_{50} (or LC_{90}) for the least effective plant/ LC_{50} (or LC_{90}) for the plant.

RESULTS

Larvicidal activity of plant oil-resin against *Cx. pipiens* (Table 1 and Fig. 1&2) indicated that mortalities were increased by increasing the concentration and exposure time. The highest larval mortalities were observed by 1500 ppm acetone extracts of *C. molmol* (83.3% and 100%) and *A. heterophylla* (75% and 95%) after 24 and 48 h PT, respectively, whereas the mortality percentage induced by the aqueous extract (1500 ppm) of *A. heterophylla* (38.3% and 55%) was more effective than that of *C. molmol* (35% and 48.3%) 24 and 48 h PT, respectively.

The LC_{50} values of the acetone extracts of *C. molmol*, *A. heterophylla*, *E. camaldulensis*, *B. sacra*, and *P. lentiscus* were 623.52, 826.03, 1110.92, 1556.50, and 2390.71 ppm 24 h PT, respectively and 300.63, 384.71, 628.65, 832.78, and 1185.69 ppm 48 h PT, respectively. While the LC_{50} of the aqueous extracts were 3178.22, 2819.85, 5431.82, 3905.87, and 7698.73 ppm 24 h PT, respectively, and 2322.53, 1652.50, 4058.74, 6857.36, and 5907.06 ppm after 48 h PT, respectively. Twenty-four hours PT, the RE values of the acetone extracts of *C. molmol*, *A. heterophylla*, *E. camaldulensis*, and *B. sacra*, were 3.8, 2.9, 2.2, and 1.5 times, respectively, more toxic than that of *P. lentiscus* as a reference substance, while those of the aqueous extracts were 2.4, 2.7, 1.4 and 2.0 times more toxic, respectively (Table 3 and 4).

The chemical constituents of *C. molmol* and *A. heterophylla* were identified by GC-MS analysis (Tables 5 & 6) indicating that *C. molmol* contains 14 main chemical compounds (terpenes, ketone, aldehyde, pesticide fatty acid, saturated fatty acid, unsaturated fatty acid, alkane, aliphatic acid, heterocyclic compound, fatty alcohol, cholesterol, fatty alcohol, phenol, and wax. *A. heterophylla* possesses six chemicals (terpenes, saturated fatty acid, phenol, alkanes, fatty alcohol, and ketone) and unknown compounds.

Most of the compounds belong to terpenes and phenol in the plants. 4,4'-Dimethyl-2,2'-dimethylenebicyclohexyl-3,3'-diene (14.62%), α -pinene (11.42%), cis-Verbenol (6.84%), n-Hexadecanoic acid (6.20%) and 2-PENTANONE, 4-HYDROXY-4-METHYL- (5.13%) were the most abundant chemical compounds in *C. molmol*. Copaene (13.64%), α -Guaiene (10.32%), trans-Verbenol (7.20%), D-Limonene (7.06%), and Caryophyllene oxide (6.62%) were the most abundant chemicals in *A. heterophylla*.

DISCUSSION

Plants contain secondary metabolites inducing antimicrobials and insecticidal effects (Khater 2013; Khater *et al.*, 2018). Except for *Commiphora sp.*, the other oil-resin plants were applied against *Cx. pipiens* for the first time.

The present study indicated that acetone extracts (1500 ppm) of *C. molmol* (83.3%, 100%) and *A. heterophylla* (75%, 95%) were the most effective ones 24 and 48 h PT. Similarly, *Commiphora sp.* oil-resin was recorded to have larvicidal activity against *Cx. pipiens* (Baranitharan *et al.*, 2017; Muturi *et al.*, 2020) and some other mosquito spp. (Mkangara *et al.* 2015). Furthermore,

Table 1: Larvicidal activity of plant oil-resin extracts against 4th larval instar *Cx. pipiens* 24 hours post-treatments

Conc. (ppm)	Mean Mortality % ± SE									
	<i>Commiphora molmol</i>		<i>Araucaria heterophylla</i>		<i>Eucalyptus camaldulensis</i>		<i>Boswellia sacra</i>		<i>Pistacia lentiscus</i>	
	Acetone	Aqueous	Acetone	Aqueous	Acetone	Aqueous	Acetone	Aqueous	Acetone	Aqueous
Control	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}
75	8.3±1.67 ^{eB}	0±0 ^{fB}	5.0±0.0 ^{fB}	0±0 ^{fB}	3.3±1.7 ^{eB}	0±0 ^{eB}	3.3±1.7 ^{eB}	0±0 ^{fA}	1.7±1.6 ^{eB}	0±0 ^{fA}
150	15.0±1.7 ^{eB}	5.0±0.0 ^{eB}	11.7±4.4 ^{eB}	5.0±0.0 ^{eB}	6.7±1.6 ^{eB}	3.3±1.6 ^{deB}	6.7±1.6 ^{deB}	3.3±1.7 ^{eB}	3.3±1.7 ^{dB}	1.6±1.7 ^{dA}
300	23.3±6.0 ^{dB}	10.0±2.9 ^{dB}	23.3±4.4 ^{dB}	11.7±1.7 ^{dB}	15.0±2.9 ^{dB}	6.7±4.4 ^{dB}	11.6±1.7 ^{dB}	8.3±1.6 ^{dB}	6.6±1.6 ^{dB}	3.3±1.7 ^{dB}
600	43.0±2.8 ^{CB}	16.7±3.3 ^{CB}	36.7±4.4 ^{CB}	18.3±1.7 ^{CB}	25.0±2.9 ^{CB}	11.7±4.4 ^{CB}	16.7±1.6 ^{CB}	13.3±1.7 ^{CB}	13.3±1.7 ^{CB}	8.3±4.4 ^{CB}
1200	65.0±4.4 ^{bB}	25.0±2.9 ^{bB}	50.0±5.7 ^{bB}	28.3±6.0 ^{bB}	46.6±4.4 ^{bB}	16.7±3.3 ^{bB}	40.0±2.9 ^{bB}	20.0±2.9 ^{bB}	26.7±4.4 ^{bB}	13.3±1.6 ^{bB}
1500	83.3±4.4 ^{aB}	35.0±5.0 ^{aB}	75.0±5.0 ^{aB}	38.3±4.4 ^{aB}	66.7±6.0 ^{aB}	26.7±4.4 ^{aB}	58.3±4.4 ^{aB}	31.7±1.6 ^{aB}	45.0±7.2 ^{aB}	18.4±3.3 ^{aB}

a, b, c, d: Means within the same column have the same small letters and means within the same row have the same capital letters are not significantly different (P>0.05, LSD).

Table 2: Larvicidal activity of plant oil-resin extracts against 4th larval instar *Cx. pipiens* 48 hours post- treatments

Conc. (ppm)	Mean Mortality % ± SE									
	<i>Commiphora molmol</i>		<i>Araucaria heterophylla</i>		<i>Eucalyptus camaldulensis</i>		<i>Boswellia sacra</i>		<i>Pistacia lentiscus</i>	
	Acetone	Aqueous	Acetone	Aqueous	Acetone	Aqueous	Acetone	Aqueous	Acetone	Aqueous
Control	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}	0±0 ^{fA}
75	11.6±1.6 ^{fA}	6.6±1.6 ^{fA}	11.6±1.6 ^{fA}	6.6±1.6 ^{eA}	8.3±3.3 ^{fA}	3.3±1.6 ^{dA}	6.7±1.6 ^{eA}	1.6±1.6 ^{fA}	3.3±1.6 ^{fA}	1.6±1.6 ^{dA}
150	25.0±3.3 ^{eA}	11.6±4.4 ^{eA}	18.3±1.6 ^{eA}	6.6±1.6 ^{eA}	15.0±2.8 ^{eA}	6.6±4.4 ^{dA}	11.6±4.4 ^{eA}	8.3±1.6 ^{eA}	8.3±1.6 ^{eA}	3.3±1.6 ^{dA}
300	46.7±6.0 ^{dA}	20.0±2.8 ^{dA}	40.0±5.7 ^{dA}	18.3±1.6 ^{dA}	25.0±2.8 ^{dA}	13.3±3.3 ^{cA}	18.3±3.3 ^{dA}	15.0±2.8 ^{dA}	18.3±4.4 ^{dA}	10.0±2.8 ^{cA}
600	70.0±7.2 ^{cA}	25.0±2.8 ^{cA}	58.3±7.3 ^{cA}	25.0±2.8 ^{cA}	40.0±5.0 ^{cA}	21.6±1.6 ^{bA}	26.6±4.4 ^{cA}	21.6±1.6 ^{cA}	28.3±4.4 ^{cA}	15.0±0.0 ^{bA}
1200	95.0±4.4 ^{bA}	33.3±4.4 ^{bA}	78.3±3.3 ^{bA}	38.3±4.4 ^{bA}	65.0±5.7 ^{bA}	25.0±2.8 ^{bA}	58.3±4.4 ^{bA}	28.3±1.6 ^{bA}	45.0±2.8 ^{bA}	18.3±1.6 ^{bA}
1500	100±0.0 ^{aA}	48.3±6.0 ^{aA}	95.0±1.6 ^{aA}	55.0±5.7 ^{aA}	85.0±4.4 ^{aA}	35.0±2.8 ^{aA}	73.3±5.7 ^{aA}	43.3±4.4 ^{aA}	63.3±3.3 ^{aA}	28.3±1.6 ^{aA}

a, b, c, d: Means within the same column have the same small letters and means within the same row have the same capital letters are not significantly different (P>0.05, LSD).

Table 3: Lethal concentrations and relative efficacy of the oil-resin extracts against 4th mosquito larvae after 24 h.

Plant oil-resin	Solvents	LC ₅₀ (95%CL)	RE	LC ₉₀ (95%CL)	Slope ±SD	χ ²
<i>Commiphora molmol</i>	Acetone	623.52 (499.13-734.19)	3.8	3441.63 (2550.56-5126.61)	1.7274±0.1391	8.2566*
	Aqueous	3178.22 (2173.00-5833.66)	2.4	28689.07 (12855.73-110990.14)	1.3412±0.1790	0.9761*
<i>Araucaria heterophylla</i>	Acetone	826.03 (707.19-1011.21)	2.9	5337.17 (3540.28-9027.19)	1.6063±0.1376	8.3825*
	Aqueous	2819.85 (1883.43-4336.01)	2.7	21477.86 (10579.99-67879.93)	1.4026±0.1754	0.9046*
<i>Eucalyptus camaldulensis</i>	Acetone	1110.92 (913.46-1381.37)	2.2	6184.13 (4280.26-9800.03)	1.7359±0.1610	6.7745*
	Aqueous	5431.82 (3177.08-14413.78)	1.4	56774.11 (19522.48-425992.24)	1.2575±0.2005	1.3629
<i>Boswellia sacra</i>	Acetone	1556.50 (1130.38-3332.74)	1.5	9979.47 (6730.12-58799.17)	1.5883±0.1652	9.6317
	Aqueous	3905.87 (2544.29-8035.02)	2.0	34470.71 (14339.65-161577.68)	1.3551±0.1947	1.7887*
<i>P. lentiscus</i>	Acetone	2390.71 (1792.69-3676.25)	-	15188.22 (8257.58-40288.41)	1.5657±0.1934	5.7199*
	Aqueous	7698.73 (3984.86-30000.58)	-	74754.17 (21572.66-1050322.45)	1.2982±0.2417	0.2799*

*P<0.05; significant level; LC: Lethal concentrations; RE: relative efficacy.

Table 4: Lethal concentrations and relative efficacy of the oil-resin extracts against 4th mosquito larvae after 48 h

Plant oil-resin	Solvents	LC ₅₀ (95%CL)	RE ¹	LC ₉₀ (95%CL)	Slope ±SD	χ ²
<i>Commiphora molmol</i>	Acetone	300.63 (262.73-344.02)	3.9	11150.65 (924.94-1527.73)	2.1986±0.1726	4.4453*
	Aqueous	2322.53 (1572.00-4287.72)	2.5	43049.92 (16658.58-215255.25)	1.0107±0.1361	3.1474*
<i>Araucaria heterophylla</i>	Acetone	384.71 (268.74-546.76)	3.1	1704.91 (1289.27-3411.53)	1.9411±0.1396	10.4385
	Aqueous	1652.50 (1251.67-2431.84)	3.6	16727.72 (8787.13-45177.54)	1.2748±0.1441	3.6478*
<i>Eucalyptus camaldulensis</i>	Acetone	628.65 (437.52-978.08)	1.9	3389.40 (2481.67-9075.62)	1.7453±0.1412	11.9340
	Aqueous	4058.74 (2450.85-9634.54)	1.5	72076.40 (23644.20-535339.55)	1.0258±0.1521	1.2758*
<i>Boswellia sacra</i>	Acetone	832.78 (542.02-1442.92)	1.4	4537.11 (4116.67-21927.49)	1.6380±0.1453	12.8368
	Aqueous	6857.36 (3628.87-22642.35)	0.9	118070.47 (32048.19-1486745.72)	1.0369±0.1718	2.7898*
<i>Pistacia lentiscus</i>	Acetone	1185.69 (974.17-1519.27)	-	7722.41 (4986.38-14457.18)	1.5731±0.1515	3.8638*
	Aqueous	5907.06 (3273.08-17423.81)	-	89610.14 (26677.19-896416.57)	1.0833±0.1745	2.2053*

*P<0.05; significant level; LC: Lethal concentrations; RE: relative efficacy.

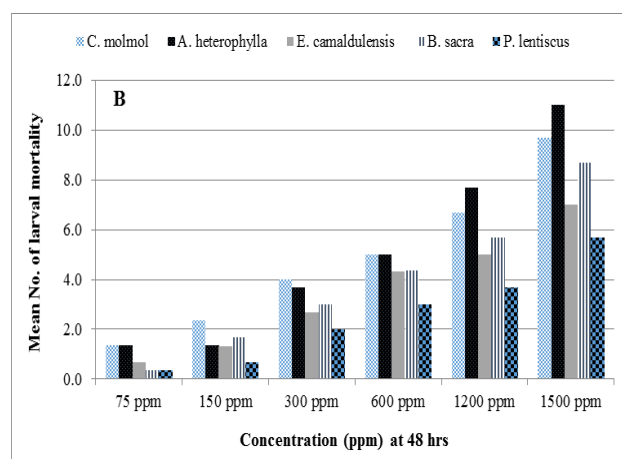
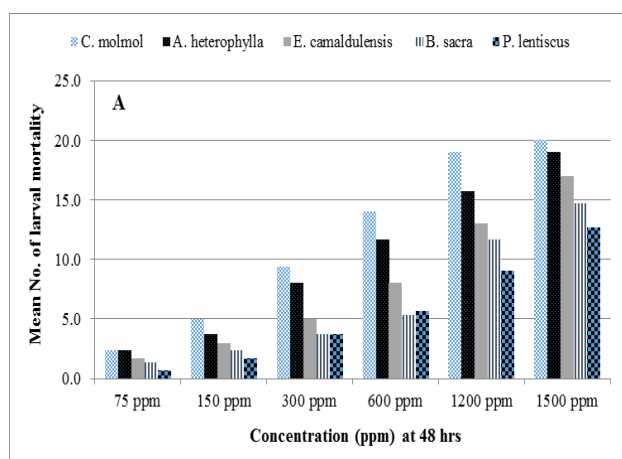


Fig. 1: Mean number of larval mortalities induced by the acetone (A) and aqueous (B) extracts of the oil-resins of *Commiphora molmol*, *Araucaria heterophylla*, *Eucalyptus camaldulensis*, *Boswellia sacra*, and *Pistacia lentiscus* against 4th larval instars, 48 h post- exposure.

Table 5: Chemical constituents of essential plant resin-oil derived from *Commiphora molmol*

No.	Chemical name (99.6%)	RT	Peak area (%)	Molecular Formula	Nature of compound
1	2-PROPANONE	5.34	2.17	C ₃ H ₆ O	Aliphatic ketone
2	2-PENTANONE, 4-HYDROXY-4-METHYL-	8.43	5.13	C ₆ H ₁₂ O ₂	Hydroxy ketone
3	Succindialdehyde	9.89	0.82	C ₄ H ₆ O ₂	Aldehyde
4	Acetaldehyde, O-methyloxime C ₈ H ₁₄ O	10.32	1.26	C ₃ H ₇ NO	Aldehyde
5	1-Hepten-6-one, 2-methyl-	13.03	0.55	C ₈ H ₁₄ O	ketone
6	Copaene	15.02	2.23	C ₁₅ H ₂₄	Terpenes
7	α-pinene	15.11	11.42	C ₁₀ H ₁₆	Terpenes
8	β-pinene	15.86	1.81	C ₁₀ H ₁₆	Terpenes
9	2-Heptanone, 6-methyl-	16.12	0.34	C ₈ H ₁₆ O	Aliphatic ketone
10	Z-3-Methyl-2-hexenoic acid	17.38	0.84	C ₇ H ₁₂ O ₂	Aliphatic acid
11	7-epi-cis-sesquibabinene hydrate	23.62	2.20	C ₁₅ H ₂₆ O	Terpene
12	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2à,4à)]-	25.94	3.38	C ₁₅ H ₂₄	Terpenes
13	9-OCTADECENOIC ACID (Z)-	26.72	0.60	C ₁₈ H ₃₄ O ₂	Fatty acid
14	ç-Elementene	27.43	1.01	C ₁₅ H ₂₄	Terpenes
15	1-DODECENE	29.39	1.08	C ₁₂ H ₂₄	Alkanes
16	Ethyl iso-allocholate	38.91	3.13	C ₂₆ H ₄₄ O ₅	Pesticide fatty acid
17	Retinal	29.57	0.49	C ₂₀ H ₂₈ O	Terpenes
18	à-acorenenol	30.31	1.76	C ₁₅ H ₂₆ O	Terpenes
19	cis-Verbenol	30.53	6.84	C ₁₀ H ₁₆ O	Phenol
20	Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-, trans-	30.71	4.62	C ₁₅ H ₂₀ O	HCC
21	ç-Muuroleone	30.11	4.48	C ₁₅ H ₂₄	Terpenes
22	PHENOL, BIS(1,1-DIMETHYLETHYL)-	31.41	0.65	C ₁₄ H ₂₂ O	Phenol
23	Dodecanoic acid	32.98	3.90	C ₁₂ H ₂₄ O ₂	SFA
24	Methyl stearidonate	34.07	0.65	C ₁₉ H ₃₀ O ₂	FAME
25	Cubanol	34.61	1.07	C ₁₅ H ₂₆ O	Terpene
26	9-OCTADECENOIC ACID (Z)-	34.79	2.18	C ₁₈ H ₃₄ O ₂	USFA
27	4,4'-Dimethyl-2,2'-dimethylenebicyclohexyl-3,3'-diene	35.54	14.62	C ₁₆ H ₂₂	Terpenes
28	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	36.22	0.95	C ₁₆ H ₂₆ O ₂	SFA
29	Cholestan-3-ol, 2-methylene-, (3à,5à)-	36.48	1.91	C ₂₈ H ₄₈ O	Cholesterol
30	1-Heptatriacotanol	37.66	2.52	C ₃₇ H ₇₆ O	Fatty alcohol
31	Hexadecanoic acid, methyl ester	38.50	3.13	C ₁₇ H ₃₄ O ₂	FAME
32	Ethyl iso-allocholate	38.91	3.43	C ₂₆ H ₄₄ O ₅	Pesticide fatty acid
33	n-Hexadecanoic acid	39.18	6.20	C ₁₆ H ₃₂ O ₂	SFA
34	OXIRANEOCTANOIC ACID, 3-OCTYL-, CIS-	40.91	0.96	C ₁₈ H ₃₄ O ₃	SFA
35	Dodecyl cis-9,10-epoxyoctadecanoate	41.03	1.24	C ₃₀ H ₅₈ O ₃	Wax

SFA: Saturated fatty acid, USFA: Unsaturated fatty acid, FAME: fatty acid methyl ester; HCC: Heterocyclic compound.

Table 6: Chemical constituents of essential plant resin-oil derived from *Araucaria heterophylla*

No.	Chemical name (98.4%)	RT	Peak area (%)	Molecular Formula	Nature of compound
1	BICYCLO [3.1.1]HEPT-2-ENE, 2,6,6-TRIMETHYL-	8.74	4.02	C ₁₀ H ₁₆	Phenol
2	à-Pinene	9.40	3.24	C ₁₀ H ₁₆	Phenol
3	D-Limonene	13.06	7.06	C ₁₀ H ₁₆	Terpenes
4	cis-p-mentha-1(7),8-dien-2-ol	16.67	1.95	C ₁₀ H ₁₆ O	Terpenes
5	Camphenol, 6-	17.70	0.89	C ₁₀ H ₁₆ O	Phenol
6	Isopinocarveol	18.19	2.68	C ₁₀ H ₁₆ O	Phenol
7	trans-Verbenol	18.49	7.20	C ₁₀ H ₁₆ O	Phenol
8	3-Tridecene, (E)-	20.90	3.28	C ₁₃ H ₂₆	Alkanes
9	n-Hexadecanoic acid	21.12	4.50	C ₁₆ H ₃₂ O ₂	SFA
10	(-)-MYRTENOL	21.52	0.53	C ₁₀ H ₁₆ O	Terpenes
11	D-Verbenone	23.05	3.12	C ₁₀ H ₁₄ O	Ketone
12	.alfa.-Copaene	23.79	2.25	C ₁₅ H ₂₄	Terpenes
13	à-Cubebene	24.13	1.11	C ₁₅ H ₂₄	Terpenes
14	à-ylangene	24.79	2.18	C ₁₅ H ₂₄	Terpenes
15	Copaene	25.09	13.64	C ₁₅ H ₂₄	Terpenes
16	(-)-à-Bourbonene	25.58	4.19	C ₁₅ H ₂₄	Terpenes
17	Caryophyllene	27.60	2.03	C ₁₅ H ₂₄	Terpenes
18	Aromandendrene	27.71	1.88	C ₁₅ H ₂₄	Terpenes
19	ç-Muuroleone	29.21	5.40	C ₁₅ H ₂₄	Terpenes
20	Germacrene D	29.84	1.74	C ₁₅ H ₂₄	Terpenes
21	à-Muuroleone	30.05	2.44	C ₁₅ H ₂₄	Terpenes
22	à-Guaiene	31.47	10.32	C ₁₅ H ₂₄	Terpenes
23	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)	32.73	1.82	C ₂₁ H ₃₄ O ₂	Fatty acid
24	Caryophyllene oxide	34.01	6.62	C ₁₅ H ₂₄ O	Phenol
25	Epiglobulol	35.76	1.65	C ₁₅ H ₂₆ O	Phenol
26	1-Heptatriacotanol	37.75	2.66	C ₃₇ H ₇₆ O	Fatty alcohol

the resin extract of *C. molmol* has pesticidal activity against blowfly, *Lucilia sericata* (Hoda *et al.*, 2016), and the fowl tick *Argas persicus* (Massoud *et al.*, 2005). A biochemical study showed that myrrh, *C. molmol* (oil, and oleoresin) can change cell proteins and decline enzyme activity in *Cx. pipiens* larvae (Massoud *et al.*, 2001).

This work indicated that both extracts of *C. molmol* and *A. heterophylla* were the most efficient ones, three to four times more effective than that of *P. lentiscus*, 48 h PT and revealed also that they have terpenes and phenol. Similarly, the efficacy of *C. molmol* and *A. heterophylla* oil-resins may be due to the presence of secondary

metabolites as contains terpenes, sesquiterpenes, cuminic aldehyde, and eugenol (Cao *et al.*, 2019) and α -pinene, limonene, and α -terpinolene found in *Bacopa caroliniana* essential oil showed good AChE inhibitory action against rice weevils (Liu *et al.*, 2020). Likewise, the oil resin of *Pinus sylvestris* (Conifers) has larvicidal toxicity against *Aedes aegypti* and *Culex quinquefasciatus* with more than 85% larval mortality after 24 h and its main constituent is alpha-terpineol (Fayemiwo *et al.*, 2014).

A. heterophylla is the second-best candidate for this study, similarly, the gum Arabic, *Acacia senegal* has larvicidal activity (Daffalla, 2018). To enhance the mosquito larvicidal activity, the gum polysaccharide of *A. heterophylla* and *Azadirachta indica* were utilized for encapsulation of cyfluthrin loaded superparamagnetic iron oxide nanoparticles (Samrot *et al.*, 2020).

E. camaldulensis has moderate larvicidal efficacy in the present work, but eucalyptus oil is a weak fly attractant and whereas p-Menthane-3,8-diol (PMD, from *Corymbia citriodora*, Family: Myrtaceae) is a strong fly repellent (Khater and Geden, 2019) and has been used in commercial mosquito repellents (Khater *et al.*, 2019).

Similar to that of *E. camaldulensis*, *B. sacra* has a moderate larvicidal efficacy shown in this work, in the same token, *Boswellia serrata* had similar efficacy against *Cx. pipiens* (Khater and Shalaby, 2008); whereas the efficacy of *Boswellia dalzielii* extract act as mosquito larvicide, ovicide, and pupicide against *An. gambiae* and *Cx. quinquefasciatus* and n-hexane fraction of *B. dalzielii* might be used as a mosquitocidal agent in the breeding sites (Younoussa *et al.*, 2016).

The lowest larval mortalities in the present work were recorded for the acetone extracts of *P. lentiscus*. Likewise, a significant effect of *P. lentiscus* on 4th instar larvae of *Cx. pipiens* increased with a combination of the essential oils of *Mentha microphylla* and *Myrtus communis* (Traboulsi *et al.*, 2002); *P. lentiscus* acts as a fumigant agent against *Tribolium castaneum* and *Lasioderma serricornis* only at higher concentration and longer exposure times (Bachrouh *et al.*, 2010).

Some studies have praised the efficacy of the plant resin extracts and have prompted more studies are needed to be directed towards developing the Conifer resins derived products through nanoformulations that enhance the efficacy to minimize the number of applications (Govindarajan *et al.*, 2016a,b; Samrot *et al.*, 2020).

Conclusions: The use of local plant oil-resins as a natural insecticide is of immense significance because of the environmental and toxicological implications of the nonstandard indiscriminate use of synthetic pesticides and for reducing the problem of increasing pest resistance.

For the first time, according to our knowledge, the oil-resin plants *A. heterophylla*, *E. camaldulensis*, and *B. sacra* were evaluated as larvicides against *Cx. pipiens* and the efficacy of *Commiphora molmol* were compared to that of the other oil-resin plants. This work highlighted the potential larvicidal effects of the acetone extracts (1500 ppm) of *C. molmol* and *A. heterophylla* and revealed their chemical constituents.

Further studies are recommended for developing nanoformulations of the conifer resins to enhance their efficacy and to minimize the number of applications after testing their ecotoxicological profiles.

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