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RESEARCH ARTICLE

Efficacy and Comparative Toxicity of Phytochemical Compounds Extracted from Aromatic Perennial Trees and Herbs against Vector Borne *Culex pipiens* (Diptera: Culicidae) and *Hyalomma dromedarii* (Acari: Ixodidae) as Green Insecticides

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A B S T R A C T

The control of vector-borne diseases with pesticides is becoming a real challenge due to rapid development of insecticide resistance. The study aimed to find out the efficacy of phytochemical compounds found in perennial aromatic trees (*Araucaria (A.) heterophylla, Eucalyptus (E.) camaldulensis,* and *Pinus (P.) halepensis*) or herbs (*Cyperus (C.) rotundus, Mentha (M.) arvensis,* and *Rosmarinus (R.) officinalis*) as natural insecticides against *Culex (Cx.) pipiens* and *Hyalomma (H.) dromedarii*. Methanol and hexane plant leaves and resin oils were extracted by Soxhlet extraction methods, separately. These compounds are specialized metabolites that are synthesized in environments with high or low pressure, depending on growing conditions and plant type. Data showed that the aromatic perennial tree, *A. heterophylla* (100%MO, LC_{50} = 90.47 ppm), and the herb *R. officinalis* (100%MO, LC_{50} = 110.56 ppm), are having activity against *Cx. pipiens*, while we found that Aleppo pine, *P. halepensis* (100%MO, LC_{50} = 1.95 mg/mL), and the herb *M. arvensis* (100%MO, LC_{50} = 2.25 mg/mL) were among the best essential oils against ticks, *H. dromedarii*, 24 hours post-treatment. The results confirmed that the diversity of phytochemicals found in aromatic perennial trees and herbs, such as sesquiterpene (á-ylangene, 23.26%), monoterpene (Eucalyptol, 22.15%), fatty acid (Linoleic acid, 19.54%), sesquiterpene (α-cyperone, 21.12%), phenol (Menthyl acetate, 15.14%), flavonoids (Bornyl acetate, 8.18%), and other active biological phytochemical compounds. The current findings indicated that aromatic pine trees essential oils, methanol extract in general, and hexane extract were the best at controlling pests and distinctive in containing phytochemicals.

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INTRODUCTION

Vector-borne diseases are still a big concern for public health all over the world, especially in tropical and subtropical areas. In addition, there are more than three billion people residing in infected areas, which poses a threat to public health. Arthropod vectors can spread many dangerous pathogens, so they can spread many infectious diseases that affect animals and humans alike (Gubler, 2010; Burkett-Cadena and Vittor, 2018; Socha *et al.*, 2022).

Many illnesses can spread directly from person to person, they can when certain factors come together, such as interactions between viruses, hosts, vectors, and susceptible populations (Abbas *et al.*, 2014; WHO, 2021; Chala and Hamde, 2021).

Although chemical pesticides are considered good at stopping the spread of diseases spread by insects, they are the most used in control programs. Excessive and repeated use of pesticides often results in environmental pollution, interferes with the food web, and is highly toxic (Gyawali, 2018). In addition to the technical and operational reasons, as well as the increasing resistance of mosquitoes and ticks to chemical pesticides and the lack of awareness due to socio-economic reasons, mosquito and tick densities have increased in many developing countries, including Egypt.

Bioactive phytochemicals, pheromones and microorganisms like bacteria, fungi, viruses, or protozoa that are all natural sources of bio-insecticides are safe alternatives to chemical pesticides (Prabha *et al.,* 2016). As people become more conscious of environmental health and safety issues, bio-sourced products that are ecologically friendly and safe to use are growing in popularity in many facets of our lives (Baz *et al.,* 2022a; Şengül Demirak and Canpolat, 2022). Many agencies and researchers are seeking to explore new biologically active phytochemicals in many plants and herbs and the extent of their toxicity in suppressing diseases or controlling disease vectors. It has been demonstrated that plant essential oils (EOs) and resin-oil are possible substitutes for synthetic insecticides against mosquito and ticks' vectors (Pavela 2015; Abbas *et al.,* 2018; Baz *et al.,* 2021).

The essential resin-oil derived from *A. heterophylla* possesses several therapeutic qualities, such as anticancer, antiviral, antibacterial, anti-inflammatory, bioinsecticidal, antiulcerative, neuroprotective, and anti-depressant effects (Elshamy *et al.,* 2020; Younis *et al.,* 2022; Baz *et al.,* 2022b). The essential oil of *A. heterophylla* contains many compounds or classes of chemicals, including diterpenes and sesquiterpenes, flavonoids, phenolic acids and polysaccharides (Abd-ElGawad *et al.,* 2023). Furthermore, *A. heterophylla* resin-oil has industrial applications as well as insecticidal properties against mosquitos and other insects (da Silva *et al.,* 2013; Samrot *et al.,* 2020; Abd-ElGawad *et al.,* 2023). Mentha essential oil showed high larvicidal activity and an excellent ability to repel *Cx. pipiens* and *Aedes aegypti* mosquitoes at different concentrations (Mohafrash *et al.,* 2020; Abbas *et al.,* 2022). Essential oils are also promising natural larvicides for controlling mosquitoes and many parasites (Al-Hoshani *et al.,* 2023; Çalışkan and Emin, 2023). The powerful aroma of spearmint has a sweet nature with a menthol undertone. The oil composition of this substance comprises carvacrol, carvone, menthol, methyl acetate, limonene, and the menthone compound.

This work focuses on exploring whether these bioactive phytochemicals are more present in perennial flowering plants than in herbs and which of these plants are more toxic for disease suppression or vector control. So that we can help in pest control programs by providing a proven scientific recommendation on the necessity of using these plants in vector-borne disease control when they have proven their effectiveness.

In the present study, we aim to evaluate six essential oils and their leaves extract from *A. heterophylla, E. camaldulensis, P. halepensis, M. arvensis, O. majorana,* and *Rosmarinus officinalis* aromatic flowering plants and herbs grown in Egypt and determine the larvicidal activity of these EOs against mosquitoes, *Cx. pipiens* and camel ticks, *H. dromedarii*.

MATERIALS AND METHODS

Mosquito colony: All experiments used *Cx. pipiens* larvae acquired from the Department of Entomology, Faculty of Science, Benha University, Egypt. under laboratory conditions (27±2°C, 75-80% RH, and a 12:12 h (L/D) photoperiod). The collected larvae were raised in enamel pans measuring 25x20x10cm. These pans were filled with 2L of de-chlorinated water and provided with fish food (Tetramin®) and grinded dog biscuits, every 2 days. Adults were provided with 8-10% sucrose solution as food source. Adult and Larvae were maintained under the same laboratory conditions (Baz, 2013).

Tick collections: The camel tick, *H. dromedarii* (Acari: Ixodidae), was obtained from nearby areas around a population of afflicted camels at the slaughterhouse located in Benha, Qalyubiya governorate, Egypt. In generals, ticks were collected form animals that did not treated with pesticides.

Collection of plant materials: Resin-oils and leaves of *A. heterophylla* Salisb. (Araucariaceae), *E. camaldulensis* (Myrtaceae), and *P. halepensis* Mill. (Pinaceae) were collected from different open areas in the gardens of the Faculty of Agriculture, Benha University, Egypt. The nursery plantation of the Medicinal and Aromatic Plants Research, Faculty of Agriculture, Benha University, Egypt, where the leaves of *C. rotundus* L. (Cyperaceae), *M. arvensis* L. (Lamiaceae), and *R. officinalis* L. (Lamiaceae) were collected. Plants and herbs were identified at the Flora and Phytotaxonomic Section, Agricultural Research Center, Giza, Egypt (Fig. 1).

Plant extract preparation: The leaves of *A. heterophylla, E. camaldulensis, P. halepensis, C. rotundus, M. arvensis,* and *R. officinalis* were washed under water and subsequently allowed to air dry. Depending on the kind of solvent, stock solutions and resin oils were grinded mechanically (40–50g) with an electric stainless-steel mixer for extraction. The extracted powder were then added in Soxhlet apparatus (4–7 hours) with methanol and hexane. The filtration of the mixture was done filter paper (Whatman No. 1) and dried for 12 hours at 28°C. The extracts were then kept at -5°C in a dark bottle for a duration of 24 hours prior to the start of the experiment.

***Culex pipiens* bioassays:** Larval instars of *Cx. pipiens* were subjected to larvicidal activity treated with EOs and plant extracts, according to the protocols by WHO (2005). 20 mosquitoes with 4^{th} instar larvae were put in a 250-mL glass beaker with 62.5, 125, 250, 500, 1000, and 1500 ppm of different concentrations. The experiments were performed five times for *Cx. pipiens* larvae.

Camel tick bioassays: The camel ticks were treated with 0.8, 1.6, 3.1, 6.3, 12.5, and 25 mg/mL concentrations through the envelope technique (Zahir *et al.,* 2010). A Whatman filter paper No. 1 having 125mm diameter, were impregnated with 10 mL of EOs and plant extracts solution for 3 min and after treatment, transport to a petri dish (7.5 x 7.5cm). Three replicates (each containing ten tick larvae) were used for each concentration. Distilled water was used to impregnate the control envelopes. The envelopes were folded and fastened using a metallic clip,

Fig. 1: Leaves of *Araucaria heterophylla* (a), *Eucalyptus camaldulensis* (b), and *Pinus halepensis* (c) trees, and *Mentha arvensis* (d), *Rosmarinus officinalis* (e), and *Cyperus rotundus* (f) herbs.

with distinguishing marks such as the tested solution and concentration. Mortalities of *Cx. pipiens* and *H. dromedarii* were observed after 24 & 48 hours of post-treatment (PT) at 28±2°C and a relative humidity of 80%.

GC/MS analysis of the volatile content: Extract solution and oils from promising plants were analyzed by GC-MS along with Agilent mass spectrometry (Ashmawy *et al.*, 2018). TG-5MS fused silica capillary columns with film thicknesses of 0.1 mm, 0.251 mm, and 30 m were used. The instrument was a Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS. For GC/MS detection, an electron ionization apparatus with an ionization energy of 70 eV was used. The carrier gas was helium gas, flowing at a steady 1 mL/min rate. Temperatures for the injector and MS transfer line were set at 280°C. The temperature started out at 50°C and was maintained for a duration of two minutes. It was then increased to 150°C at a rate of 7°C per minute, further to 270°C at a rate of 5°C per minute (holding for two minutes), and finally to 310°C at a rate of 3.5°C per minute (holding it for ten minutes). By contrasting their retention times and mass spectra with those in the mass spectrum databases WILEY 09 and NIST 11, the components were identified.

Data analyses: The collected data was analyzed through Duncan's multiple range tests, one-way analysis of variance (ANOVA) and Probit analyses to compute lethal concentrations. These statistical tests were conducted using the computing software PASW Statistics 2009 (SPSS version 22).

RESULTS

Effect of the essential oil and plant extracts on *Culex pipiens*: The EOs and plant extracts examined in this study showed significant insecticidal activity against mosquito larvae of *Culex pipiens* after different intervals of exposure. The mortality percent (MO%) at 24 and 48 hours post-treatment (PT) of *Cx. pipiens* with 1500 ppm essential resin-oil of *A. heterophylla*, *E. camaldulensis*, and *P. halepensis* were 100% with LC_{50} (50%, median lethal concentration) = 90.47 and 78.87; 135.66 and 110.36; and 105.70 and 90.98 ppm, respectively (Table 1 and Fig. 2a,b); whereas those of methanol extracts were 100, 100 and 98%, at 1500 ppm, 48 h PT with (LC_{50} = 258.44, 207.16 and 282.47 ppm. While in hexane extracts, mortality reached 90, 97 and 93% at 1500 ppm, 48 hours PT with (LC_{50} = 372.20, 302.90, and 401.07 ppm, respectively) (Table 1 and Fig. 2b).

Data of the larvicidal activity of *C. rotundus*, *M. arvensis*, and *R. officinalis* essential oils against *Cx. pipiens* are presented in Table 2. In terms of lethal concentrations LC_{50} for *R. officinalis* oil (100 MO%) appeared to be most effective against *Cx. pipiens* (LC_{50} = 110.56 and 87.67 ppm), followed by *C. rotundus* (LC_{50} = 140.56 and 115.78 ppm), and *M. arvensis* (LC_{50} = 190.34 and 162.92 ppm) at 1000 ppm, 24 and 48 hours PT (Table 2 and Fig. 3a,b). While at methanol extracts, the mortality reached 100, 95, and 90% at 24 hours PT with (LC_{50} = 178.57, 235.41, and 289.79 ppm) and 100% at 48 h PT with (LC_{50} = 104.07, 153.73, and 226.74 ppm) For *R. officinalis, C. rotundus and M. arvensis* extracts, respectively (Fig. 3b). Data showed that hexane extracts was less effective with LC_{50} = 145.0, 190.84, and 303.11 ppm, 48 hours PT, respectively (Fig. 3b).

Effect of the essential oil and plant extracts on *Hyalomma dromedarii*: It was evident from the results that essential resin-oil effectively controlled the camel tick, *H. dromedarii*, where 100% mortality was reached at seven days PT at 10% (mg/mL) concentrations of *A. heterophylla*, *E. camaldulensis*, and *P. halepensis* with LC_{50} = 1.23, 1.82, and 0.92%, respectively (Table 3 and Fig. 4), whereas those of methanol were 100, 96, and 100% mortality (LC_{50} = 1.87, 4.35, and 3.15), and as well as the hexane extracts were 100, 86, and 100% with (LC_{50} = 2.79, 5.81, and 4.40, respectively, at 20% concentrations, 7 days PT.

The toxicity indices for *C. rotundus, M. arvensis, and R. officinalis* methanol extracts were 96, 100, and 100% mortality at 20% concentration, 7 day PT, respectively with the LC_{50} values= 4.15, 1.75, and 2.65, respectively. Also, data shwoed that the hexane extracts effects were 84, 100, and 100%, at 20% concentration, 7 days PT with LC_{50} = 6.29, 3.27, and 4.09, respectively (Table 4 and Fig. 5).

Table 1: Efficacy of the plant resin-oil and leaves extracts of *Araucaria heterophylla, Eucalyptus camaldulensis* and *Pinus halepensis* on the mortality % of *Culex pipiens*, 24 and 48 hours post-treatment

Plant extract	Conc. (ppm)	Resin-oil		Methanol		Hexane	
		24	48	24	48	24	48
Araucaria heterophylla	0	00±0.00^{eA}	00±0.00^{eA}	00±0.00^{gA}	00±0.00^{gA}	00±0.00^{gA}	00±0.00^{gA}
	62.5	32±3.00^{dB}	37±1.22^{dA}	13±1.22^{fCD}	16±2.45^{fCD}	10±1.58^{fD}	13±2.00^{fCD}
	125	67±5.15^{cB}	74±5.57^{cA}	23±1.22^{eCD}	26±2.45^{eC}	18±2.00^{eE}	21±2.45^{eDE}
	250	88±3.39^{bB}	98±1.22^{bA}	40±3.54^{dD}	46±3.67^{dC}	31±2.92^{dE}	37±2.00^{dD}
	500	100±0.00^{aA}	100±0.00^{aA}	56±5.34^{cC}	63±4.64^{cB}	48±2.55^{cD}	55±2.74^{cC}
	1000	100±0.00^{aA}	100±0.00^{aA}	75±3.54^{bC}	86±2.92^{bB}	64±2.92b^{BD}	72±2.00^{bC}
	1500	100±0.00^{aA}	100±0.00^{aA}	94±3.67^{aB}	100±0.00^{aA}	82±2.55^{aD}	90±3.54^{aC}
Eucalyptus camaldulensis	0	00±0.00^{fA}	00±0.00^{eA}	00±0.00^{gA}	00±0.00^{gA}	00±0.00^{gA}	00±0.00^{gA}
	62.5	19±3.67^{eB}	24±1.87^{dA}	15±1.58^{fCD}	18±3.39^{fBC}	9±1.87^{fE}	13±2.00^{fD}
	125	47±4.64^{dB}	53±4.06^{cA}	28±3.39^{eD}	32±5.39^{eC}	23±2.00^{eE}	27±2.55^{eD}
	250	79±5.79^{cB}	87±4.06^{bA}	49±4.30^{dD}	54±3.32^{dC}	37±2.55^{dF}	43±4.06^{dE}
	500	91±3.67^{bB}	99±1.00^{aA}	67±5.15^{cD}	74±6.2^{cC}	51±4.85^{cF}	60±3.54^{cE}
	1000	100±0.00^{aA}	100±0.00^{aA}	83±2.55^{bC}	90±2.74^{bB}	67±2.55^{bE}	75±3.54^{bD}
	1500	100±0.00^{aA}	100±0.00^{aA}	98±1.22^{aA}	100±0.00^{aA}	86±3.32^{aB}	97±2.00^{aA}
Pinus halepensis	0	00±0.00^{fA}	00±0.00^{eA}	00±0.00^{gA}	00±0.00^{gA}	00±0.00^{gA}	00±0.00^{gA}
	62.5	26±3.32^{eB}	31±2.92^{dA}	12±2.55^{fCD}	15±3.16^{fC}	8±2.00^{fE}	11±2.45^{fDE}
	125	58±5.15^{dB}	65±4.47^{cA}	22±2.55^{eD}	28±3.39^{eC}	18±2.55^{eE}	21±1.87^{eDE}
	250	84±4.00^{cB}	93±2.55^{bA}	35±1.58^{dD}	42±3.74^{dC}	29±1.87^{dF}	36±1.00^{dE}
	500	98±1.22^{bA}	100±0.00^{aA}	53±5.15^{cC}	60±5.92^{cB}	45±2.74^{cD}	50±2.74^{cC}
	1000	100±0.00^{aA}	100±0.00^{aA}	72±4.64^{bC}	81±4.30^{bB}	60±3.16^{bE}	67±3.00^{bD}
	1500	100±0.00^{aA}	100±0.00^{aA}	90±2.74^{aB}	98±2.00^{aA}	85±3.54^{aC}	93±2.55^{aB}

a, b & c: In the same column with same superscript letter, Non-significant difference (P>0.05) among any two means. A, B & C: In the same row with same superscript letter, Non-significant difference (P>0.05) among any two means for the same solvent and in the same row with same superscript letter.

Table 2: Efficacy of the plant essential oil and leaves extracts of *Cyperus rotundus, Mentha arvensis, and Rosmarinus officinalis* on the mortality % of *Culex pipiens*, 24 and 48 hours post-treatment

Plant extract	Conc. (ppm)	Essential oil		Methanol		Hexane	
		24	48	24	48	24	48
Cyperus rotundus	0	00±0.00^{fA}	00±0.00^{fA}	00±0.00^{gA}	00±0.00^{fA}	00±0.00^{gA}	00±0.00^{gA}
	62.5	22±3.00^{eB}	26±1.87^{eA}	16±1.87^{fC}	22±1.87^{eB}	10±1.58^{fD}	20±2.74^{fB}
	125	48±4.36^{dB}	54±2.92^{dA}	27±2.55^{eE}	40±3.39^{dC}	22±1.22^{eF}	35±4.85^{eD}
	250	68±3.74^{cB}	76±1.87^{cA}	44±1.87^{dE}	65±3.32^{cC}	37±3.00^{dF}	57±3.00^{dD}
	500	86±4.30^{bB}	97±2.00^{bA}	69±3.32^{cE}	85±3.32^{bC}	61±2.92^{cF}	80±2.24^{cD}
	1000	100±0.00^{aA}	100±0.00^{aA}	95±2.24^{bB}	100±0.00^{aA}	75±2.24^{bC}	96±2.45^{bB}
	1500	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	95±2.24^{aB}	100±0.00^{aA}
Mentha arvensis	0	00±0.00^{fA}	00±0.00^{fA}	00±0.00^{gA}	00±0.00^{fA}	00±0.00^{gA}	00±0.00^{gA}
	62.5	16±1.87^{eB}	20±1.58^{eA}	11±1.87^{fC}	15±2.74^{eB}	9±1.87^{fC}	11±1.87^{fC}
	125	28±2.55^{dB}	32±3.00^{dA}	21±1.87^{eC}	28±2.00^{dB}	16±1.87^{eD}	21±2.55^{eC}
	250	56±2.45^{cB}	63±2.55^{cA}	41±2.92^{dD}	50±5.15^{cC}	29±1.87^{dE}	42±3.67^{dD}
	500	84±3.67^{bB}	91±4.30^{bA}	66±2.55^{cD}	77±2.55^{bC}	55±3.54^{cF}	64±3.67^{cE}
	1000	100±0.00^{aA}	100±0.00^{aA}	90±3.54^{bB}	99±1.00^{aA}	80±3.54^{bC}	88±3.67^{bB}
	1500	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	90±3.54^{aB}	100±0.00^{aA}
Rosmarinus officinalis	0	00±0.00^{fA}	00±0.00^{dA}	00±0.00^{fA}	00±0.00^{eA}	00±0.00^{gA}	00±0.00^{fA}
	62.5	26±3.32^{eC}	33±3.39^{cA}	18±2.00^{eE}	29±1.87^{dB}	13±2.55^{fF}	22±2.00^{eD}
	125	56±4.85^{dB}	64±5.57^{bA}	35±3.54^{dE}	52±6.52^{cC}	26±1.87^{eF}	41±5.34^{dD}
	250	80±5.00^{cC}	99±3.32^{aA}	55±2.74^{cE}	89±1.87^{bB}	42±3.74^{dF}	63±3.39^{cD}
	500	98±2.00^{bAB}	100±0.00^{aA}	84±2.92^{bC}	100±0.00^{aA}	67±3.39^{cD}	96±2.45^{bB}
	1000	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	86±1.87^{bC}	100±0.00^{aA}
	1500	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}

a, b & c: In the same column with same superscript letter, Non-significant difference (P>0.05) among any two means. A, B & C: In the same row with same superscript letter, Non-significant difference (P>0.05) among any two means for the same solvent and in the same row with same superscript letter.

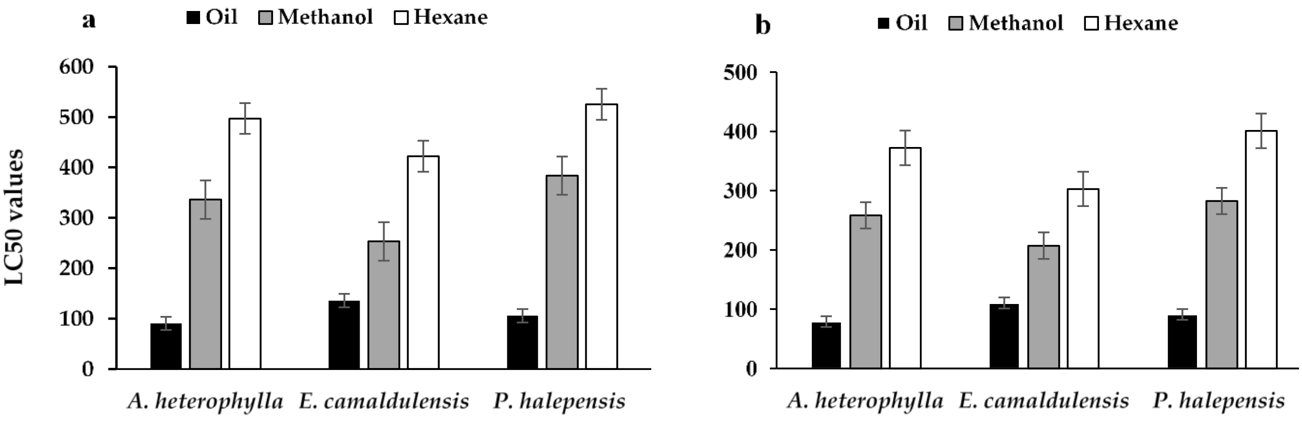

Fig. 2: The mean number of larval mortalities induced by the effects of plant extracts of *Araucaria heterophylla, Eucalyptus camaldulensis,* and *Pinus halepensis* against 3rd larval instars *Culex pipiens*, 24 (a) and 48 (b) hours post-exposure.

Table 3: Efficacy of the plant resin-oil and leaves extracts of *Araucaria heterophylla, Eucalyptus camaldulensis* and *Pinus halepensis* on the mortality % of *Hyalomma dromedarii*, 7 days post-treatment

Plant extract	Conc. (mg/mL)	Essential oil			Methanol			Hexane		
		1	3	7	1	3	7	1	3	7
	0	0±0^{gB}	0±0^{fB}	6±2.45^{eA}	0±0^{gC}	2±2.00^{gB}	6±2.45^{fA}	0±0^{fC}	2±2.00^{gB}	4±2.45^{gA}
	0.8	8±3.74^{fC}	14±2.45^{eB}	24±5.10^{dA}	4±2.45^{fC}	8±3.74^{fB}	16±2.45^{eA}	2±2.00^{fC}	6±4.00^{fB}	8±2.00^{fA}
Araucaria	1.25	24±4.00^{eC}	36±4.00^{dB}	60±5.48^{cA}	12±3.74^{eC}	22±2.00^{eB}	40±4.47^{dA}	8±3.74^{eC}	14±2.45^{eB}	26±4.00^{eA}
heterophylla	2.5	46±4.00^{dC}	68±3.74^{cB}	86±6.00^{bA}	20±3.16^{dC}	32±8.00^{dB}	62±3.74^{cA}	16±5.10^{dC}	24±5.10^{dB}	50±3.16^{dA}
	5	76±5.10^{cC}	96±4.00^{bB}	100±0.00^{aA}	42±5.83^{cC}	52±5.83^{cB}	90±4.47^{bA}	38±5.83^{cC}	40±10.00^{cB}	74±2.45^{cA}
	10	88±4.90^{bB}	100±0.00^{aA}	100±0.00^{aA}	66±4.00^{bC}	84±5.10^{bB}	100±0.00^{aA}	54±5.10^{bC}	70±6.32^{bB}	90±4.47^{bA}
	20	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	88±4.90^{aC}	90±5.48^{aB}	100±0.00^{aA}	80±6.32^{aC}	90±6.32^{aB}	100±0.00^{aA}
	0	0±0^{gB}	0±0^{fB}	6±2.45^{fA}	0±0^{fC}	2±2.00^{fB}	6±2.45^{gA}	0±0^{fB}	0±0^{fB}	4±2.45^{fA}
	0.8	4±2.45^{fC}	10±3.16^{eB}	18±7.35^{eA}	0±0.00^{fC}	4±2.45^{fB}	10±3.16^{fA}	0±0.00^{fC}	2±2.00^{fB}	4±2.45^{fA}
	1.25	16±2.45^{eC}	28±3.74^{dB}	40±6.32^{dA}	4±2.45^{eC}	8±3.74^{eB}	18±3.74^{eA}	4±2.45^{eC}	6±4.00^{eB}	10±4.47^{eA}
Eucalyptus camaldulensis	2.5	26±2.4^{5dC}	50±3.16^{cB}	66±6.78^{cA}	10±3.16^{dC}	16±2.45^{dB}	32±1.41^{dA}	10±3.16^{dC}	18±3.74^{dB}	26±5.10^{dA}
	5	52±3.74^{cC}	68±3.74^{bB}	88±3.74^{bA}	30±3.16^{cC}	38±3.74^{cB}	58±3.74^{cA}	26±5.10^{cC}	32±4.90^{cB}	52±2.00^{cA}
	10	62±8.60^{b}B	100±0.00^{aA}	100±0.00^{aA}	46±2.45^{bC}	54±4.00^{bB}	78±3.74^{bA}	42±3.74^{bC}	50±3.16^{bB}	70±4.47^{bA}
	20	94±4.00^{aB}	100±0.00^{aA}	100±0.00^{aA}	70±5.48^{aC}	78±2.00^{aB}	96±2.45^{aA}	62±3.74^{aC}	70±6.32^{aB}	86±4.00^{aA}
	0	0±0^{fB}	0±0^{eB}	6±2.45^{dA}	0±0^{fC}	2±2^{fB}	6±2.45^{fA}	0±0^{fB}	0±0^{gB}	4±2.45^{fA}
	0.8	10±3.16^{eC}	20±3.16^{dB}	38±3.74^{cA}	0±0.00^{fC}	4±2.45^{fB}	8±3.74^{fA}	0±0.00^{fC}	2±2.00^{fB}	4±2.45^{fA}
Pinus	1.25	32±7.35^{dC}	58±4.90^{cB}	82±2.00^{bA}	8±3.74^{eC}	12±3.74^{eB}	22±3.74^{eA}	6±2.45^{eC}	8±3.74^{eB}	18±3.74^{eA}
halepensis	2.5	60±4.47^{cC}	86±5.10^{bB}	100±0.00^{aA}	12±3.74^{dC}	16±2.45^{dB}	42±2.00^{dA}	8±3.74^{dC}	12±3.74^{dB}	32±4.90^{dA}
	5	88±3.74^{bC}	100±0.00^{aA}	100±0.00^{aA}	30±3.16^{cC}	44±6.00^{cB}	76±5.10^{cA}	20±3.16^{cC}	34±5.10^{cB}	60±3.16^{cA}
	10	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	54±5.10^{bC}	66±5.10^{bB}	88±4.90^{bA}	46±4.00^{bC}	58±8.60^{bB}	74±4.00^{bA}
	20	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	80±5.48^{aC}	90±7.75^{aB}	100±0.00^{aA}	70±4.47^{aC}	80±7.07^{aB}	94±4.00^{aA}

a, b & c: In the same column with same superscript letter, Non-significant difference (P>0.05) among any two means. A, B & C: In the same row with same superscript letter, Non-significant difference (P>0.05) among any two means for the same solvent and in the same row with same superscript letter.

Table 4: Efficacy of the plant essential oil and leaves extracts of *Cyperus rotundus, Mentha arvensis, and Rosmarinus officinalis* on the mortality % of *Hyalomma dromedarii*, 7 days post-treatment

Plant extract	Conc. (mg/mL)	Essential oil			Methanol			Hexane		
		1	3	7	1	3	7	1	3	7
	0	0±0^{gC}	2±2.00^{eB}	6±2.45^{fA}	0±0^{fC}	2±2.00^{gB}	4±2.45^{gA}	0±0^{fB}	2±2.00^{gA}	2±2.00^{gA}
	0.8	2±2.00^{fC}	4±2.45^{eB}	10±3.16^{eA}	0±0^{fC}	4±2.45^{fB}	10±3.16^{fA}	0±0^{fB}	4±0.00^{fA}	4±2.45^{fA}
	1.25	12±4.9^{eC}	22±2.00^{dB}	38±5.83^{dA}	4±2.45^{eC}	8±3.74^{eB}	18±3.74^{eA}	2±2.00^{eC}	6±2.45^{eB}	10±4.47^{eA}
Cyperus rotundus	2.5	22±3.74^{dC}	44±4.00^{cB}	76±4.00^{cA}	6±2.45^{dC}	16±2.45^{dB}	32±3.74^{dA}	8±2.00^{dC}	14±2.45^{dB}	20±4.47^{dA}
	5	54±5.10^{cC}	70±4.47^{bB}	96±2.45^{bA}	26±5.10^{cC}	38±3.74^{cB}	58±3.74cA	22±3.74^{cC}	30±3.16^{cB}	48±4.9^{cA}
	10	88±5.83^{bB}	100±0.00^{aA}	100±0.00^{aA}	42±5.83^{bC}	54±4.00^{bB}	78±3.74^{bA}	38±3.74^{bC}	46±2.45^{bB}	66±7.48^{bA}
	20	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	66±5.10^{aC}	78±2.00^{aB}	96±2.45^{aA}	58±3.74^{aC}	68±5.83^{aB}	84±5.10^{aA}
	0	0±0^{fC}	4±2.45^{eB}	6±2.45^{dA}	0±0^{gC}	2±2.00^{gB}	4±2.45^{fA}	0±0^{fC}	2±2.00^{gB}	4±2.45^{gA}
	0.8	8±3.74^{eC}	16±2.45^{dB}	30±3.16^{cA}	4±2.45^{fC}	8±3.74^{fB}	16±2.45^{eA}	0±0^{fC}	4±2.45^{fB}	8±2.00^{fA}
	1.25	24±5.10^{dC}	44±5.10^{cB}	82±6.63^{bA}	8±3.74^{eC}	18±4.90^{eB}	40±4.47^{dA}	4±2.45^{eC}	10±3.16^{eB}	18±3.74^{eA}
Mentha arvensis	2.5	54±5.10^{cC}	90±4.47^{bB}	100±0.00^{aA}	14±2.45^{dC}	32±8.00^{dB}	66±1.93^{cA}	12±3.74^{dC}	22±3.74^{dB}	34±2.45^{dA}
	5	82±3.74^{bB}	100±0.00^{aA}	100±0.00^{aA}	38±7.35^{cC}	52±5.83^{cB}	92±4.90^{bA}	34±4.00^{cC}	42±4.90^{cB}	66±5.10^{cA}
	10	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	62±2.00^{bC}	94±4.00^{bB}	100±0.00^{aA}	54±5.10^{bC}	70±5.48^{bB}	96±4.00^{bA}
	20	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	94±4.00^{aB}	100±0.00^{aA}	100±0.00^{aA}	82±4.90^{aC}	92±5.83^{aB}	100±0.00^{aA}
	0	0±0^{dC}	4±2.45^{fB}	6±2.45^{eA}	0±0^{fC}	2±2.00^{gB}	4±2.45^{gA}	0±0^{fC}	0±0^{gB}	2±2.00^{gA}
	0.8	6±4.00^{fC}	10±3.16^{eB}	20±5.48^{dA}	0±0^{fC}	4±2.45^{fB}	8±3.74^{eA}	0±0^{fC}	2±2.00^{fB}	4±2.45^{fA}
	1.25	16±5.10^{eC}	26±4.00^{dB}	56±6.78^{cA}	8±3.74^{eC}	12±3.74^{eB}	22±3.74^{dA}	4±2.45^{eC}	8±2.00^{eB}	14±5.10^{eA}
Rosmarinus officinalis	2.5	28±3.74^{dC}	58±3.74^{cB}	82±4.90^{bA}	12±3.74^{dC}	16±2.45^{dB}	42±2.00^{cA}	10±3.16^{dC}	16±5.10^{dB}	28±2.00^{dA}
	5	64±6.00^{bC}	90±3.16^{bB}	100±0.00^{aA}	26±5.10^{cC}	46±5.10^{cB}	80±3.16^{bA}	24±5.10^{cC}	38±3.74^{cB}	60±3.16^{cA}
	10	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	56±5.10^{bC}	80±7.07^{bB}	100±0.00^{aA}	48±3.74^{bC}	58±5.83^{bB}	80±5.48^{bA}
	20	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	90±4.47^{aB}	100±0.00^{aA}	100±0.00^{aA}	76±6.78^{aC}	90±6.32^{aB}	100±0.00^{aA}

a, b & c: In the same column with same superscript letter, Non-significant difference (P>0.05) among any two means. A, B & C: In the same row with same superscript letter, Non-significant difference (P>0.05) among any two means for the same solvent and in the same row with same superscript letter.

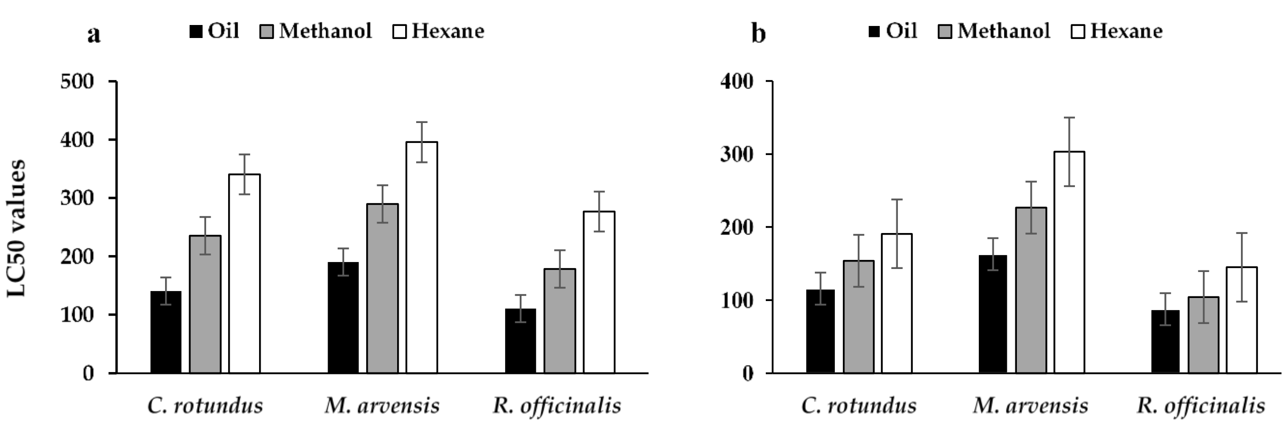

Fig. 3: The mean number of larval mortalities induced by the effects of plant extracts of *Cyperus rotundus, Mentha arvensis*, and *Rosmarinus officinalis* against 3rd larval instars *Culex pipiens*, 24 (a) and 48 (b) hours post-exposure.

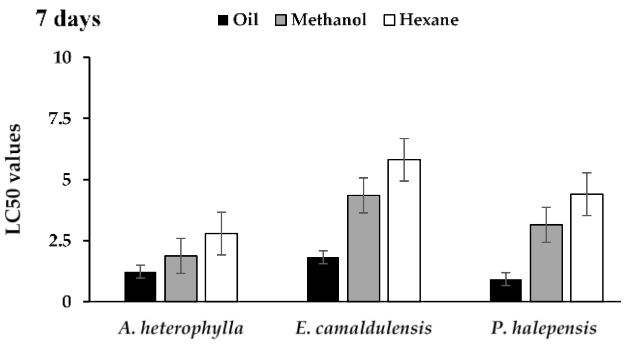

Fig. 4: The mean number of larval mortalities induced by the effects of plant extracts of *Araucaria heterophylla, Eucalyptus camaldulensis and Pinus halepensis* against ticks, *Hyalomma dromedarii*, 7 days post-exposure.

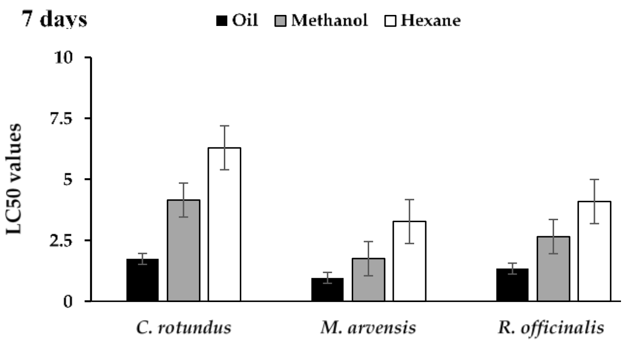

Fig. 5: *The mean number of larval mortalities induced by the effects of plant extracts of* Cyperus rotundus, Mentha arvensis, and Rosmarinus officinalis *against ticks,* Hyalomma dromedarii*, 7 days post-exposure.*

GC/MS identification of volatile content: *A. heterophylla, E. camaldulensis, P. halepensis, C. rotundus, M. arvensis,* and *R. officinalis* essential oils and plant extracts were subjected to phytochemical analysis using a GC/MS chromatogram. Identified phytochemical compounds were compared to already documented compounds in the National Institute of Standards and Technology with regard to peak retention times, peak areas (%) and mass spectral fragmentation patterns.

The relative percentages of the constituents are listed in Tables S1-S6. 126 compounds were identified from for *A. heterophylla*, *E. camaldulensis*, *P. halepensis*, *C. rotundus*, M. arvensis, and R. officinalis essential oils, respectively. The major components of each species were: á-ylangene (23.26%), à-Pinene (11.10%), 19-D-Torulosol (10.36%), cis-Verbenol (8.11%), and oleic acid (6.21%) *A. heterophylla*; Eucalyptol (22.15%), α-pinene (18.15%), 1,8-cineole (11.20%), and Hexadecanoic acid (9.26%) for *E. camaldulensis*; Linoleic acid (19.54%), Copaene (17.25%), and α-Pinene (14.12%) for P. halepensis; α-cyperone (21.12%), Caryophyllene oxide (16.14%), and Octadecane, 1-chloro- (11.14%) for *C. rotundus*; Menthone (19.51%), Menthyl acetate (15.14%), and Caryophyllene (12.11%) for M. arvensis and 1,4-Cineol (20.32%), α-Pinene (10.03%), Camphor (9.42%) and Bornyl acetate (8.18%) for *R. officinalis*.

The GC–MS analysis showed that sesquiterpene (á-ylangene, 23.26%), monoterpene (Eucalyptol, 22.15%), fatty acid (Linoleic acid, 19.54%), sesquiterpene (α-cyperone, 21.12%), phenol (Menthyl acetate, 15.14%), and flavonoids (Bornyl acetate, 8.18%) were the most major phytochemical constituents in *A. heterophylla, E. camaldulensis, P. halepensis, C. rotundus, M. arvensis,* and *R. officinalis* essential oils, respectively (Fig. S1).

DISCUSSION

The essential oils of the aromatic perennial trees and herbs *A. heterophylla* and *R. officinalis* having activity over against *Cx. pipiens*. Similar to our study, the *A. heterophylla* oil resin was highly effective against *Cx. pipiens* larvae and induced the highest larval mortalities, which were observed either in acetone and aqueous extracts.

Its clear from the literature that the essential oils of the pine plants were rarely used or applied against mosquitoes or insects in general. In a similar investigation, Fayemiwo *et al.* (2014) showed that the oil resin derived from *Pinus sylvestris* (Conifers) exhibits larvicidal properties against *Ae. aegypti* and *Cx. quinquefasciatus,* resulting in larval mortality reaching 85% during a 24-hour period. The main component of *P. sylvestris* oil is alpha-terpineol. On the other hand, we find that rosemary oil and its plant extracts (methanol and hexane) are some of the most powerful herbal selected oils on mosquito larvae. In a similar study, our results agreed with those of Bosly's (2022); their findings indicated a dose-related reaction. The rosemary oil displayed the greatest larvicidal (100%) activity at 1000 ppm (LC_{50}, 214.97 ppm).

R. officinalis essential oil demonstrated larvicidal action against fourth-stage larvae of *Culex pipiens* at 24 hours, according to a bioassay test with 100 mortality (LC_{50} = 51.33 ppm). When used at LC_{50}, EO caused a notable reduction in the morphometric parameters of the larvae and disturbed energy stores with a noticeable lipid loss at various times. Additionally, an increase in GST activity and a drop in GSH levels show how the detoxification system is stimulated by the treatment (Zeghib *et al.,* 2020). It has been demonstrated that rosemary (*R. officinalis*) oil has larvicidal effects on animals. The essential oils of *Pinus kesiya* showed significant acute toxicity on the early 3rd larvae of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*, with LC_{50} values of 52, 57, and 62 mg/mL, respectively. Notably, the EO had LC_{50} values between 4135 and 8390 mg/mL for the aquatic non-target species *Anisops bouvieri*, *Diplonychus indicus,* and *Gambusia affinis* (Govindarajan *et al.,* 2016).

Similar to the response of mosquito larvae *Cx. pipiens*, the results of this work showed that essential resin-oil effectively controlled the camel tick, *H. dromedarii*, where 100% mortality was reached at seven days PT at 10% (mg/mL) concentrations of *A. heterophylla, E. camaldulensis,* and *P. halepensis*.

Data showed that the aromatic perennial Aleppo pine, *P. halepensis*, and herb of *M. arvensis* were among the best essential oils against ticks, *H. dromedarii*. The efficiency of both oils Moroccan pine (*Pinus halepensis* Mill. And *Pinus pinaster* Sol.) was evaluated on lentil beetle, or *Bruchus signaticornis*. Data showed that the essential oils of *P. halepensis* and *P. pinaster* are very dangerous to this insect. The results of the tests revealed a significant amount of activity that was supported by the LD_{50} values, 24 hours post-treatment and was likely caused by the primary constituents. *B. signaticornis* was totally killed in the presence of the two essential oils after ten days of contact at a dose of 2.4 x $10^{-2}/cm^3$.

There are several studies on the potential sources of bioactive molecules of pine oils of different families against pests and pathogenic mealybugs, including oils (*Pinus halepensis* and *P. holdreichii*), which have been shown to have antibacterial and insect larval activity (Mitić *et al*., 2019). It was also found that pine oils are from the family Cupressaceae family (*Cupressus funebris, Juniperus chinensis,* and *Juniperus communis*) were toxic to *Ae. Aegypti* larvae and adults and if they would repel host-seeking *Amblyomma americanum* and Ixodes scapularis ticks. All of the oils repelled both kinds of ticks (Tabanca *et al.,* 2011; Salman *et al.,* 2023).

Our data showed the aromatic perennial plants had an abundance of terpenes, while herbal plants had a high abundance of phenols and flavonoids. Some of the phytochemicals found in conifer products are stilbenes, terpenes, alkaloids, lignins, flavonoids (including quercetin, rutin, and resveratrol), and the substances PYC and enzogenol. These chemicals have been linked to calming, diabetes-fighting, cancer-fighting, and anesthetic effects (Bhardwaj *et al.,* 2021).

A. heterophylla is in the genus *Araucaria*. It has a wide range of pharmacological properties, including antiulcerogenic, antibacterial, antioxidant, anticancer, and toxoplasmicidal properties (Branco and Scola, 2015; Younis *et al.*, 2022). There were 16 different chemicals found in the essential oils of both samples. The cultivated variety had the highest amounts of 1,8-cineole (32.18%), camphor (16.20%), and α-pinene (15.40%). The predominant component of the mixture in the rosemary samples obtained from the wild populations is -pinene, accounting for 51.19% of the total.

The most prevalent classes of compounds in the oil of *P. Halepensis* were sesquiterpenes and diterpenes, where €-caryophyllene and thunbergol were the main compounds (32.2 and 29.2%, respectively). The monoterpene content of the oil from *P. heldreichii* was high, with limonene (34.4%) and -pinene (23.8%) making up more than 50% of the total (Mitić *et al.,* 2019).

Coniferous phytochemical compounds have been known for a long time to have the potential to treat several diseases and be used in industry. The most prevalent natural compounds in these plants are terpenes, alkaloids, and polyphenols. According to Dolan *et al*. (2009) research, the Cupressaceae family may be a rich source of anti-tick compounds. Consequently, additional investigation into cypress species for the development of repellents and insecticidal chemicals is imperative. On the other hand, we find some herbal plants from Lamiaceae with high toxicity against many insect pests (Qureshi *et al.,* 2017; Elmhalli *et al.,* 2019; Muturi *et al.,* 2019; Iqbal *et al.,* 2022).

Conclusions: Conventional pesticides are still the main way that people around the world try to get rid of insects, but insects have become resistant to almost all types of pesticides. Because of the broad variety and high efficacy of many plant-borne compounds, botanicals as environmentally friendly pesticides represent safe and suitable alternatives. The food and cosmetic sectors, as well as the pharmaceutical, cosmetic, and insecticide industries, use natural products extensively. Our findings showed that essential oils, followed by the methanol extract and then the overall hexane extract, were the most effective at affecting pests and most notable for having secondary biological compounds. Aleppo pine, *P. halepensis*, and the herb *M. arvensis* were among the best essential oils against ticks, *H. dromedarii*. The aromatic perennial tree, *A. heterophylla*, and the herb *R. officinalis* are having action against *Cx. pipiens*.

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Authors' contributions: Conceptualization, MMB, ATA, LAA, AFM; methodology, MMB, ATA, LAA; software, ATA, MMB; validation, LAA, MMB; formal analysis, ATA, LAA, MMB, AFM; investigation, MMB, ATA, LAA; data management, MMB, ATA, AFM, LAA; writing—original draft preparation, MMB, ATA, LAA; writing—review and editing, MMB, ATA, AFM, LAA; supervision, MMB, ATA, LAA; All authors have read and agreed to the published version of the manuscript.

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