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# Assessment of the acaricidal efficacy of *Rosmarinus officinalis* essential oil against dogs' ticks, *Rhipicephalus sanguineus* (Acari: Ixodidae), and its chemical composition

*Avaliação da eficácia acaricida do óleo essencial de Rosmarinus officinalis contra carrapatos de cães, Rhipicephalus sanguineus (Acari: Ixodidae), e sua composição química*

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**ABSTRACT**

Ticks play the main role, in veterinary terms, in transmitting important pathogens. *Rhipicephalus sanguineus* is a widespread tick known for its ability to thrive in indoor domestic environments and could be the main reservoir host for many TBDs, which infest dogs living in urban areas. In this study, the acaricidal and larvicidal potential of *Rosmarinus officinalis* essential oil was evaluated against *R. sanguineus*. The aerial part of this plant was extracted by hydrodistillation and then analyzed by gas chromatography coupled with mass spectrometry (GC/MS).
The yield obtained from this oil was 0.38%, its major chemical compounds were found to be Camphor (43.52%), Eucalyptol (13.66%), and Camphene (13.2%). The adult immersion test (AIT) using four concentrations (1µl/ml,2µl/ml,10µl/ml, 30µl/ml) revealed that this oil presented oviposition reduction percentages of 5.75%, 20.68%, 33.27%, and 46.84%, hatching reductions percentages of 5%, 15%, 35%, and 60%, and efficacy extract percentages of 10.46%, 32.58%, 56.63%, and 78.74%, respectively. Further, the larval immersion test (LIT) using five concentrations (0.5µl/ml,1µl/ml, 2µl/ml, 3µl/ml, and 5µl/ml) revealed considerable larvicidal activities with LC50 and LC90 values of 2.286 µl/ml and 5.380 µl/ml, respectively. These results are encouraging and open interesting and promising horizons for its application as a bio-acaricide.

**KEYWORDS:** ticks; acaricide; essential oil; *Rosmarinus officinalis;* toxicological parameters.

**RESUMO**

Os carrapatos desempenham o papel principal, em termos veterinários, na transmissão de patógenos importantes. O Rhipicephalus sanguineus é um carrapato muito difundido, conhecido por sua capacidade de se desenvolver em ambientes domésticos e pode ser o principal hospedeiro reservatório de muitas DTAs que infestam cães que vivem em áreas urbanas. Neste estudo, o potencial acaricida e larvicida do óleo essencial de Rosmarinus officinalis foi avaliado contra o R. sanguineus. A parte aérea dessa planta foi extraída por hidrodestilação e depois analisada por cromatografia gasosa acoplada à espectrometria de massa (GC/MS). O rendimento obtido desse óleo foi de 0,38%, e seus principais compostos químicos foram a cânfora (43,52%), o eucaliptol (13,66%) e o canfeno (13,2%). O teste de imersão de adultos (AIT) usando quatro concentrações (1µl/ml, 2µl/ml, 10µl/ml, 30µl/ml) revelou que esse óleo apresentou porcentagens de redução de oviposição de 5,75%, 20,68%, 33,27% e 46,84%, porcentagens de redução de eclosão de 5%, 15%, 35% e 60% e porcentagens de eficácia do extrato de 10. 46%, 32,58%, 56,63% e 78,74%, respectivamente. Além disso, o teste de imersão de larvas (LIT) usando cinco concentrações (0,5 µl/ml, 1 µl/ml, 2 µl/ml, 3 µl/ml e 5 µl/ml) revelou atividades larvicidas consideráveis com valores de LC50 e LC90 de 2,286 µl/ml e 5,380 µl/ml, respectivamente. Esses resultados são encorajadores e abrem horizontes interessantes e promissores para sua aplicação como bioacaricida.

**PALAVRAS-CHAVE:** carrapatos; acaricida; óleo essencial; Rosmarinus officinalis; parâmetros toxicológicos.

## INTRODUCTION

Ticks are obligate hematophagous ectoparasitic arthropods that depend entirely on one or more hosts to complete their life cycle; they are the most widespread arthropod, with more than 900 species worldwide (MANS & NEITZ 2004). Hard ticks (Ixodidae) are the dominant family of ticks, considering the number of species and their veterinary and medical importance (TSATSARIS et al. 2016).

The danger of these arachnids lies in their ability to transmit important pathogens (protozoa, bacteria and viruses) during bites (SONENSHINE et al. 2002). This transmission occurs through 3 routes: transstadial (from one life stage to another through molting), horizontal (through a host and during co-feeding) and transovarial transmission (from an infected female to her progeny). The latter (TOT) is the most important in maintaining the existence of a variety of pathogens (including *Rickettsia spp*. and Babesia spp. and many viruses). What makes ticks a reservoir of harmful vector-borne diseases (AZAD & BEARD 1998, BALASHOV 1999, BONNET et al. 2007, DANIELOVÁ et al. 2002).

The brown tick, *Rhipicephalus sanguineus* (LATREILLE 1806 – ROMA et al. 2013), is mainly an ectoparasite of dogs but is frequently associated with other animals, including humans, as hosts (SCHUSTER et al. 2009, KABIR et al. 2011, MENTZ et al. 2016). *R. sanguineus* is involved in the transmission of different etiological agents, such as Babesia canis, Ehrlichia canis, and Rickettsia conorii, which are the etiological agents of canine babesiosis, canine monocytic ehrlichiosis, and Mediterranean spotted fever, respectively (BRUMPT 1932, GROVES et al. 1975, REGENDANZ & MUNIZ 1936).

Unlike other species of exophilic ticks that live in open environments, pastures, or forests (PAROLA & RAOULT 2001), *Rhipicephalus sanguineus* is endophilic, known for its ability to thrive in indoor environments; the engorged female separated from the domestic dog can lay eggs in the residence (USPENSKY & IOFFE-USPENSKY 2002). Due to the high reproductive rate of ticks, their population can increase rapidly in a short period, resulting in severe residential infestation (KOCH 1982).

For a long time, the control of these arthropods was based on the use of synthetic acaricides, which offer relatively rapid and effective control of tick populations. The use of these chemical pesticides often results in many more problems than can be solved (SAVADOGO et al. 2016). The intensive and continuous application of ticks on the host and its surroundings creates toxicity problems for animals and humans, leading to environmental pollution and the development of tick resistance (DANDE 2015).

To reduce this dilemma, it becomes necessary to focus on natural plant compounds (ABDELALI et al. 2023) as essential oils that have been widely used in various fields (AISSAOUI et al. 2022). Furthermore, research on acaricidal plants in veterinary parasitology is a recent field of research worldwide; however, in Algeria, little work has been done in this context (ALIMI et al. 2022, DJEBIR et al. 2019).

Algeria is known for its richness in medicinal plants, considering its surface area and bioclimatic diversity (GHOMARI et al. 2014), among them *Rosmarinus officinalis* (Iklil in Arabic), which is a species of flowering plant of the Lamiaceae family, exists in the Mediterranean region and grows wild in Algeria, France, Italy, Portugal, Morocco, and Spain, while it is cultivated in several countries such as the United States (VERMA et al. 2012). It is commonly used as a condiment and food preservative consisting of bioactive molecules and phytocompounds that are responsible for several pharmacological activities, such as anti-inflammatory activities (OLIVEIRA et al. 2019).

The aim of this study was to determine the chemical composition of the essential oil of the local plant *Rosmarinus officinalis* and evaluate the effects of different concentrations on larval mortality and reproductive aspects of females of R. sanguineus, initiating a biological control using an environmentally friendly and less harmful natural substance.

## MATERIAL AND METHODS

### Extraction of plant and essential oil

The aerial parts of *Rosmarinus officinalis* (Figure 1) were collected in May in the Djebal Hawas region (34◦ 41' N, 3 ◦ 09'02" E) in Djelfa, determined by comparison with a sample from the herbarium of the Missouri Botanical Garden, voucher number (3844178). The identification was confirmed by Mr. A. Brague, Chief Forest Inspector of the National Forestry Research Institute of Djelfa Province. The plant leaves were initially rinsed with distilled water and dried in the shade at room temperature. Next, 50 g of plant powder was hydrodistilled for 3 h using a Clevenger-type apparatus (CLEVENGER 1928) according to the recommendations of the Hellenic Pharmacopoeia (HELLENIC PHARMACOPOEIA 2002). The essential oil was dissolved in diethyl ether, dried over anhydrous magnesium sulfate $MgSO_4$ and stored in hermetically sealed sterile glass bottles, protected from light, at a temperature of 4 ºC, until gas chromatographic analysis and toxicological study.

The essential oil yield was estimated using the formula given by FALLEH et al. (2008):

R (%) = (Mext / M´ech.)  100.

Here, R is the yield in %. Mext is the mass of the extract (in g) after evaporation of the solvent. M´ech is the dry mass of the plant sample (in g).

Figure 1. *Rosmarinus officinalis*.

### Chemical analysis

The chemical composition of the essential oil was analyzed by gas chromatography coupled to mass spectrometry (GC/MS), which allowed the qualitative and quantitative determination of most of the compounds in the sample (2-5 µl). The essential oil was transferred to a gas chromatograph vial, diluted in hexane (1-2 ml), and sealed with a high-performance septum (DELAZAR et al. 2004). Constituents were identified by comparing their mass spectra with those stored in the NIST/EPA/NIH mass spectral database (version 2.0 as of May 19, 2011).

***Rhipicephalus sanguineus***

Engorged females of *R. sanguineus* were collected from naturally infested domestic dogs shortly after they began to abandon the host to ensure uniformity. These hosts did not receive any acaricide treatment for at least 45 days to avoid any negative interference in many farms in the municipality of Ain Maabed (34° 48′ 17″ N, 3° 07′ 46″ E), Djelfa, Algeria.

Ticks were stored in cooled plastic boxes (≈15°C) to reduce their activity and immediately transported to the laboratory, where they were carefully washed with distilled water and dried on paper towels. Species were identified using binocular magnifying glass according to the keys and descriptions provided by WALKER et al. (2003).

**Preparation of the toxicological test**

This test was performed in two stages: engorged females and larvae, using an immersion test (AIT/LIT). *Rosmarinus officinalis* essential oil was dissolved and serially diluted in 1 ml of ethanol. Preliminary tests with different doses were performed to select a range of concentrations before starting the toxicity test. Four concentrations (1 µl/ml, 2 µl/ml, 10 µl/ml, 30 µl/ml) were chosen for AIT, and five concentrations (0.5 µl/ml, 1 µl/ml, 2 µl/ml, 3 µl/ml, 5 µl/ml) for LIT. For each concentration, three replicates were maintained, as in the control.

**Adult immersion test**

AIT was performed as described in the literature (DRUMMONDS et al. 1973, FAO 2004) with minor modifications. In groups of fifteen engorged female ticks, each was weighed individually to obtain groups with similar weights (0.5±0.1 g). The different groups of ticks were immersed in 10 ml of each concentration for 5 min. All tests were replicated three times. After exposure, engorged females were removed, dried, and placed in Petri dishes, which were incubated for 15 days at 27 ± 2 °C and 80% relative humidity. Tick death was confirmed based on signs of hemorrhagic skin lesions, cuticular darkening, and absence of Eustachian tube movement. After 2 weeks, the eggs were weighed, transferred to tubes, and placed in an incubator under the same hatching conditions as the larvae.

The egg production index (EPI), hatching reduction (RE), oviposition reduction (RO), reproductive efficiency index (REI), and extract efficiency (EP) were calculated using the following formulas:

EPI (%) = (weight of eggs/weight of engorged female) × 100 (BENNETT 1974)

RO (%) = [(EPI control group− EPI experimental group)/EPI control group] × 100 (ROULSTON et al. 1968)

HR (%) = [(hatching rate in control group-hatching rate in experimental group) / hatching rate in control group] × 100 (GONZALES 2003)

REI = (egg mass weight × % egg hatch/weight of engorged females) × 20,000 (DRUMMONDS et al. 1973)

EP (%) = [(REI control − REI treated)/REI control] × 100 (DRUMMONDS et al. 1973)

**Larval immersion test (LIT)**

LIT is not recommended or standardized by FAO. Therefore, the following protocol was modified from a previous test described by (RIBEIRO et al. 2011). The larvae used in this test (LIT) were from eggs provided by untreated engorged females; larval treatments were performed on the 15th day after total larval hatching.

A number of 100 larvae were immersed for 5 min in tubes containing 10 mL of different concentrations of *Rosmarinus officinalis* essential oil. The tubes were closed, shaken vigorously for a few seconds, and then gently shaken for 5 min.

The larvae were then transferred with a brush to dry on a paper towel. They were then placed on filter paper (8.5 × 7.5 cm) (Whatman No. 1), which was folded and closed with clips to form a packet. The packages were incubated at 27°C–28°C and ≥ 80% relative humidity.

Live and dead larvae were counted after 24 h, 48 h, and 72 h of exposure (three packages per treatment) for subsequent calculation of LC50, LC90, LT50, and LT90 for each group.

**Statistical analysis**

The mortality values obtained at the different concentrations were considered averages. These results were subjected to probit analysis to calculate lethal concentrations and lethal times (LC50% LC90%, LT50% and LT90%). This analysis was performed using IBM SPSS Statistics23 software on Windows.

## RESULTS

**Yield and chemical composition of** essential oils from ***Rosmarinus officinalis***

The oil yield of *Rosmarinus officinalis* was 1.49%. The chemical composition by GC-MS (Table 1) revealed 50 compounds with a total percentage of 100%. Five main components were identified:

Camphor (43.52%), Eucalyptol (13.66%), Camphene (13.2%), α-Pinene (8.9%), endo-Borneol (4.32%), Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)- (4.16%), β-Pinene (2.29%), and the other proportions ranging from 1.85% to 0.01% (Table 1, Figure 2).

**Acaricidal effects of *Rosmarinus officinalis*** essential oil:

All tested concentrations of *Rosmarinus officinalis* essential oil showed considerable efficiency from 10.76% to 78.74%, which resulted in a significant reduction in the egg mass of engorged females from 33.82% to 16.41% with a significant reduction in the reproductive efficiency index compared to the control group.

As a result, the egg production of *R. sanguineus* was reduced by a ratio of 5.75% to 46.84% from the minimum to maximum concentration.

In addition, a high proportion of egg hatching inhibition was obtained using this essential oil at a maximum concentration of 60% egg hatching; however, the newly hatched larvae did not survive and died within a few hours after hatching (Table 2).

Table 1. Abundance (%) of *Rosmarinus officinalis* essential oil components determined using gas chromatography-electron impact mass spectrometry (GC-MS).

| No. | RT | Compound name | Abundance % |
|---|---|---|---|
| 1 | 7.27 | α-Pineno | 8.90 |
| 2 | 8.35 | Camphene | 13.20 |
| 3 | 9.29 | β-Pinene | 2.29 |
| 4 | 9.671 | Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene, (1S)- | 0.03 |
| 5 | 9.856 | α-Phellandrene | 0.16 |
| 6 | 10.851 | Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)- | 4.16 |
| 7 | 11.571 | Eucalyptol | 13.66 |
| 8 | 12.387 | γ-Terpinene | 0.38 |
| 9 | 13.042 | Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2β,5α)- | 0.05 |
| 10 | 13.427 | Cyclohexene, 1-methyl-4-(1-methylethylidene)- | 0.46 |
| 11 | 13.662 | 4-Terpinenyl acetate | 0.03 |
| 12 | 14.157 | 1,6-Octadien-3-ol, 3,7-dimethyl- | 0.08 |
| 13 | 14.328 | exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol | 0.06 |
| 14 | 14.868 | Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, (1R-endo)- | 0.10 |
| 15 | 15.073 | Fenchol, exo- | 0.01 |

| | | | |
|---|---|---|---|
| 16 | 15.308 | exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol | 0.08 |
| 17 | 16.608 | Camphor | 43.52 |
| 18 | 17.374 | endo-Borneol | 4.32 |
| 19 | 17.789 | Terpinen-4-ol | 1.85 |
| 20 | 18.154 | Benzenometanol, α,α,4-trimetil- | 0.09 |
| 21 | 18.444 | α-Terpineol | 1.85 |
| 22 | 18.764 | (-)-Myrtenol | 0.05 |
| 23 | 19.229 | 2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, | 0.02 |
| 24 | 19.745 | D-Verbenone | 0.13 |
| 25 | 21.56 | 2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)- | 0.03 |
| 26 | 22.746 | Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester | 0.61 |
| 27 | 23.001 | Cyclohexene, 2-ethenyl-1,3,3-trimethyl- | 0.02 |
| 28 | 23.181 | Thymol | 0.03 |
| 29 | 23.431 | Phenol, 2-methyl-5-(1-methylethyl)- | 0.10 |
| 30 | 23.906 | 6-Methyl-cyclodec-5-enol | 0.04 |
| 31 | 24.987 | trans-p-Mentha-2,8-dienol | 0.05 |
| 32 | 25.867 | Phenol, 2-methoxy-3-(2-propenyl)- | 0.04 |
| 33 | 26.142 | α-ylangene | 0.03 |
| 34 | 26.532 | alpha.-Copaene | 0.14 |
| 35 | 27.288 | Geranyl isovalerate | 0.01 |
| 36 | 27.918 | Methyleugenol | 0.06 |
| 37 | 28.523 | Caryophyllene | 0.65 |
| 38 | 28.893 | β-copaene | 0.02 |
| 39 | 29.984 | Humulene | 0.13 |
| 40 | 30.609 | 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)- | 0.01 |
| 41 | 31.904 | α-Muurolene | 0.06 |
| 42 | 32.165 | β-Bisabolene | 0.04 |
| 43 | 32.51 | γ-Muurolene | 0.36 |

| | | | |
|---|---|---|---|
| 44 | 32.855 | Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- | 0.31 |
| 45 | 33.735 | 9-Methoxycalamenene | 0.02 |
| 46 | 35.371 | (-)-Spathulenol | 0.55 |
| 47 | 35.631 | 5-Hepten-3-one, 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl, [2S-[2α(R*),5α]]- | 0.41 |
| 48 | 37.332 | Cubedol | 0.04 |
| 49 | 38.142 | Cyclopentaneacetic acid, 3-oxo-2-(2-pentenyl)-, methyl ester, [1α,2α(Z)]- | 0.23 |
| 50 | 39.387 | α-Bisabolol | 0.52 |
| Total | | | 100 |

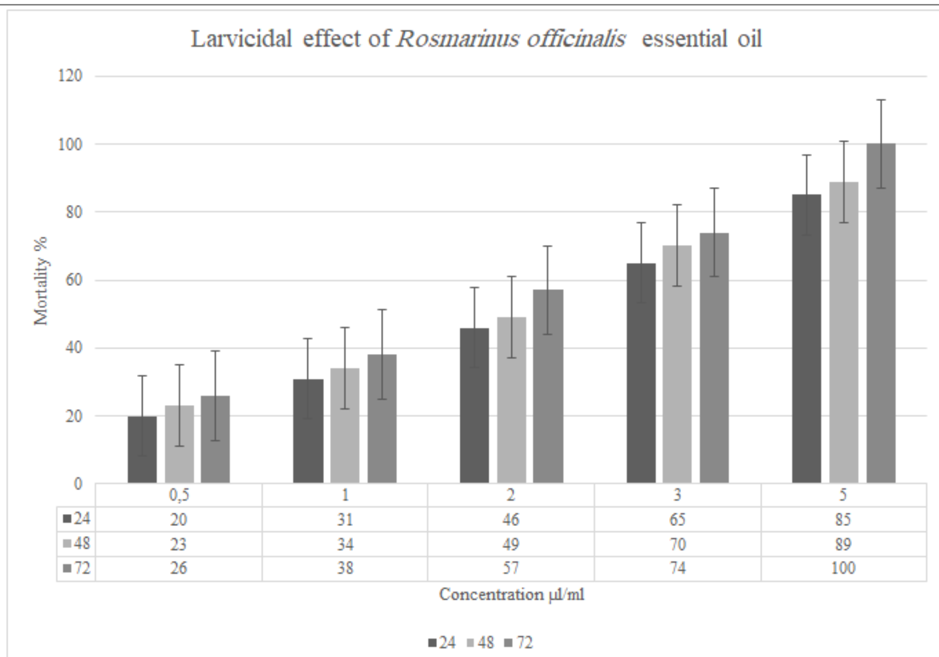


Figure 2. Chromatographic profile of *Rosmarinus officinalis* essential oil analyzed by GC-SM.

Table 2. Effects of Rosmarinus officinalis essential oil on the reproductive characteristics of *R. sanguineus* females.

| Concentration µl/ml | EPI (%) | RO (%) | KING | EP (%) | Hatching (%) | HR (%) |
|---|---|---|---|---|---|---|
| 1 | 33.82 | 5.75 | 642579.37 | 10.46 | 95 | 5 |
| 2 | 25.15 | 20.68 | 427529.76 | 32.58 | 85 | 15 |
| 10 | 20.44 | 33.27 | 265659.4 | 56.63 | 65 | 35 |
| 30 | 16.41 | 46.84 | 131256.16 | 78.74 | 40 | 60 |
| Control | 32.27 ± 1.23 | 0 | 645394.34 ± 24527.54 | 0 | 100 ± 0 | 0 |

On the other hand, the essential oil *of Rosmarinus officinalis* showed a larvicidal effect against R. sanguineus larvae, with a mortality rate that varied between 20% after 24 h for the lowest concentration (0.5 µl/ml) and up to 100% after 72 h when the larvae were exposed to the highest concentration (5 µl/ml) (Figure 3). This efficiency increased as the exposure time and oil concentration increased; furthermore, the correlation coefficients R recorded in Table 3 confirm this strong positive correlation between the recorded mortality rates and the exposure time and/or the essential oil concentration.

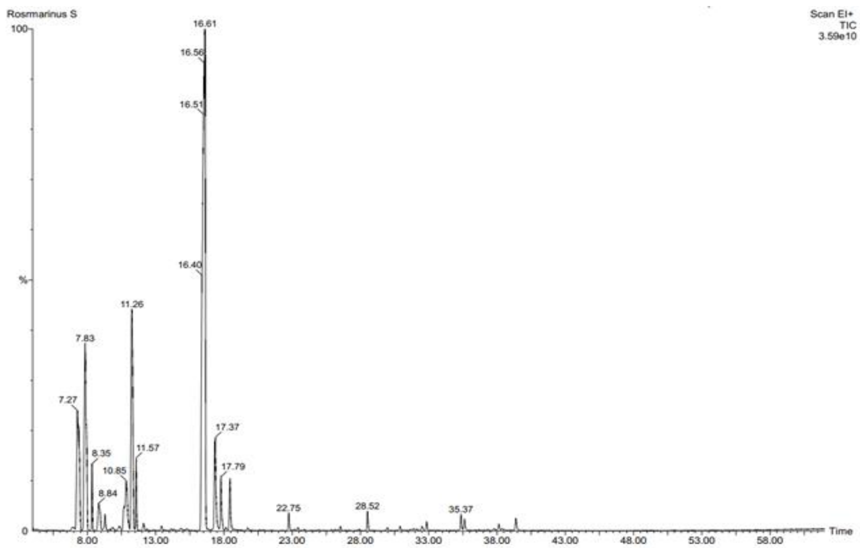


Figure 3. Evolution of the mortality rate of *R. sanguineus* larvae treated with different concentrations of Rosmarinus officinalis essential oil

Table 3. Toxicological parameters of *Rosmarinus officinalis* essential oil in R. sanguineus larvae.

A

| Time (hours) | 24 | 48 | 72 |
|---|---|---|---|
| Regression line | E = -0.95+0.41x | E = -0.88+0.43x | E = -0.85+0.51x |
| CL = 50% (µl/ml) | 2.286 | 2.021 | 1.635 |
| LC 90% (µl/ml) | 5.380 | 4.935 | 3.783 |
| 95% Confidence Interval | [0.334 0.495] | [0.356 0.524] | [0.487 0.706] |
| Chi-square value | 1.416 | 1.109 | 4.45 |
| P-value | 0.702 | 0.775 | 0.217 |
| **R** | 0.988 | 0.991 | 0.995 |

B

| Concentration (µl/ml) | 0.5 | 1 | 2 | 3 | 5 |
|---|---|---|---|---|---|
| Regression line | Y=-0.94+413E-3x | Y=-0.59+3.97E-3x | Y=-0.26+5.77E-3x | Y=0.26+5.38E-3x | Y=0.85+7.92E-3x |
| LT50% (hours) | 227.572 | 149.898 | 45.076 | * | * |
| LT90% (hours) | 538.028 | 472.656 | 267.411 | 189.917 | 38.561 |
| 95% Confidence Interval | [-0.004 0.012] | [-0.003 0.011] | [-0.001 0.015] | [-0.001 0.013] | [-0.002 0.013] |
| Chi-square value | 0 | 0.006 | 0.005 | 0.168 | 0.004 |
| P-value | 0.983 | 0.941 | 0.946 | 0.682 | 0.95 |
| R | 1 | 0.995 | 0.935 | 0.998 | 1 |

*Calculation was not performed.

After 24 h, a concentration of 2,286 µl/ml guarantees 50% mortality of the larval stage; furthermore, to guarantee 90% mortality, the concentration of *R. officinalis* must be equal to 5,380 µl/ml. After 48 and 72 h of treatment, the LC50% is 2.021 µl/ml and 1.635 µl/ml, respectively, whereas the LC90% is 4.935 µl/ml and 3.783 µl/ml (Table 3).

The concentrations of 0.5 µl/ml, 1 µl/ml and 2 µl/ml Rosmarinus *officinalis* eliminated 50% of the R. sanguineus population at 9.48, 6.25, and 1.88 days. Furthermore, when the five concentrations of *R. officinalis* were applied, the LT90% was 22.42 days, 19.69 days, 11.14 days, 7.91 days, and 1.6 (Table 3).

## DISCUSSION

### Yield and chemical characterization of Rosmarinus officinalis essential oil

The yield of essential oil from *R. officinalis* was 1.49%, higher than many other works carried out, noting that the yield of oil collected in Kenya was 0.59% (MWITHIGA et al. 2022), in Portugal it was 0.3–0.7% (SERRANO et al. 2002), and in Türkiye it was 0.71–0.94% (GURBUZ et al. 2016). However, it is lower compared to those collected in Algeria, where the essential oil yield in Tbessa was 1.85-2.29% (BOUTABIA et al. 2016). The chemical composition of this oil differs from those obtained by BAKKALI et al. (2018) in Morocco, where 17 compounds represented about 75.6% of the total. The main constituents are α pinene (32.64%), β humulene (8.71%), and camphene (5.95%).

The essential oil of Indian rosmarinus was also enriched with alpha-pinene (31.91%) and 1,8-cineole (14.66%). However, in France, KALOUSTIAN et al. (2002) recorded a camphor chemotype with high-level (30-45%). Furthermore, in Algeria, BOUTABIA et al. (2016) showed that 1,8-cineole is the predominant chemotype of *essential oil from Rosmarinus officinalis*. However, Lograda et al. (2013) observed that the chemical composition of rosemary essential oils collected in five regions of eastern Algeria is dominated by camphor (42.7%).

### Acaricidal effect of *Rosmarinus officinalis essential* oil:

For a long time, resource-poor farmers in Africa and Asia have practiced traditional medicine based on the use of plant materials to treat livestock endo- and ectoparasites, including ticks (MONDAL et al. 2013). The first intensive tests on acaricidal activity were launched by KHAIDAROV (1971), which evaluated 84 plants; currently, at a global level, 200 plant species have been registered for their repellent or acaricidal properties (ADENUBI et al. 2016). The orientation toward biocides is due to the abundance of secondary plant metabolites with toxicological activity, their low cost, and relatively lower toxicity to the environment and hosts (BORGES et al. 2011), in addition to the slow development of resistance due to the variability of active agents with different mechanisms of action (BALANDRIN et al. 1985, CHAGAS et al. 2002, OLIVO et al. 2009), making plant extracts a better alternative for controlling tick populations (OLIVEIRA et al. 2016).

The toxicological tests of the present study revealed a considerable and variable sensitivity of *R. sanguineus* to the essential oil of Rosmarinus officinalis, as indicated by a significant reduction in the egg mass of engorged females from 33.82% to 16.41% with a significant reduction in the reproductive efficiency index compared with the control group. Furthermore, the essential oil was also toxic to larvae, expressed by low to very high mortality rates, which correlates with increasing time from one concentration to another, with LC50 of 2.286 µl/ml for 24 h, 2.021 µl/ml for 48 h, and 1.635 µl/ml for 72 h.

In comparison with the tick species chosen for this work, DAEMON et al. (2009) and MONTEIRO et al. (2009) showed the effectiveness of thymol on the larvae and pupae *of R. sanguineus*, with a mortality rate that reached 100% at concentrations of 2% and 0.5%, respectively. However, in the case of non-engorged R. sanguineus larvae, only 37.7% mortality was recorded at a concentration of 2% thymol (DAEMON et al. 2009), besides GODARA et al. (2013) showed the in vitro efficacy of the chloroform extract obtained from *Artemisia absinthium* on adults, eggs, and larvae using the adult immersion test (AIT) causing a mortality rate of up to 93.3% with LC50 and LC95 values of 8.793% and 34.59%, the egg hatch test (EHT) reducing egg production to 85.1% with complete inhibition of hatching, and the larval packaging test (LPT) causing 100% mortality of larvae with LC50 and LC95 values of 1.11% and 2.37%.

In Algeria, few studies have investigated the control of ticks using plant extracts. There are two studies on the same species *Hyalomma scupense* revealing considerable toxic activity, the first by DJEBIR et al. (2019) evaluating the acaricidal activity of six aromatic plants belonging to the Lamiaceae and Myrtaceae families through an adult immersion test (AIT) and a larval immersion test (LIT), and the second by ALIMI et al. (2022) evaluating the acaricidal activity of *Ocimum basilicum* essential oil and its main constituents through the adult immersion test (AIT) and the larval package test (LPT).

Notably, the toxicity of different extracts of some plants is not only limited to mortality, but can also affect the fecundity and hatching rate of female eggs (ELLSE & WALL 2014), while altering the morphophysiology of some important organs (CAMARGO-MATHIAS 2018), such as the ovaries (KONIG et al. 2020), salivary glands (REMEDIO et al. 2016), and the nonganglion (ROMA et al. 2013).

However, variations between the methods used and the conditions for testing the repellent and acaricidal effects of certain plant extracts, such as the choice of test type, test duration, presence or absence of the index host, species, and stage of ticks, as well as the plant, extraction type, and solvent, made it difficult to compare studies and select the best plant species.

## CONCLUSION

In conclusion, this toxicological study showed that the distilled essential oil of *R. officinalis* has high in vitro acaricidal activity against larvae, in addition to strongly affecting the reduction of the hatching and egg-laying capacity of engorged females of R. sanguineus.

These results provide interesting horizons for its application as a potential alternative to synthetic acaricides for the control of animal ticks. However, in vivo clinical studies under practical external conditions are also necessary to validate this control strategy to standardize experimental control design, establish the correct doses to be administered to animals, and determine side effects related to phytotoxicity.

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