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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 with over 900 species worldwide (MANS & NEITZ 2004). The hard ticks (Ixodidae) are the dominant family, in view of 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 is provided by 3 routes: trans stadial (from one life stage to another through molting), horizontal (through a host and during co-feeding), and transovarial transmission (from an infected female to its progeny), the last one (TOT) is the most important in the existence maintaining of pathogens variety (including *Rickettsia spp*. and *Babesia spp*. and many viruses). Which make 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 primarily an ectoparasite of dogs, but often 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 exophilic tick species that live in open environments, grasslands or forests (PAROLA & RAOULT 2001), *Rhipicephalus sanguineus* is endophilic, known for its ability to thrive in indoor environments, the engorged female detached from the domestic dog can lay eggs in the residence (USPENSKY & IOFFE-USPENSKY 2002), and due to the high reproductive rate of the ticks, its population can increase rapidly in a shorperiodme, resulting in severe residential infestation (KOCH 1982).

For a long time, the control of these arthropods has been based on the use of synthetic acaricides, as they offer relatively rapid and effective control of tick populations. The use of these chemical pesticides has often caused many more problems than it has solved (SAVADOGO et al. 2016). Their intensive and continuous application 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).

In order to reduce this dilemma it becomes necessary to focus on natural compounds from plants (ABDELALI et al. 2023) like essential oils which have been widely used in various fields (AISSAOUI et al. 2022). Moreover, 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 its bioclimatic diversity (GHOMARI et al. 2014) among them *Rosmarinus officinalis* (Iklil in Arabic) which is a species of flowering plant in the family Lamiaceae, 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, made up of bioactive molecules, phytocompounds, liable for the implementation of several pharmacological activities, such as anti-inflammatory activities (OLIVEIRA et al. 2019).

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

MATERIAL AND METHODS

Plant material and essential oil extraction

The aerial parts of *Rosmarinus officinalis* (Figure 1) were harvested in May from the Djebal Hawas (34° 41’ N, 3° 09’02’’ E) region in Djelfa, determined by comparison to a sample from the Missouri botanical garden herbarium, voucher number (3844178). The identification was confirmed by Mr. Brague A., Principal Forest Inspector at the National Institute of Forest Research of the province of Djelfa. The plant leaves were initially rinsed with distilled water and dried in the shade at room temperature. 50 g of plant powder was hydrodistilled for three hours using a Clevenger- type apparatus (CLEVENGER 1928) according to the recommendations of the Hellenic Pharmacopoeia (HELLENIC PHARMACOPOEIA 2002). The essential oil was taken up 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 yield of essential oil was estimated using the formula given by FALLEH et al. (2008):

R (%) = (Mext / M´ech.) * 100

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 with mass spectrometry (CG/MS), which allows both a qualitative and quantitative determination of the majority of compounds of the sample (2-5 µl), the essential oil was transferred to a GC vial, diluted in hexane (1-2 ml), and sealed with a high- performance septum (DELAZAR et al. 2004). The constituents were identified comparing their mass spectra to spectra stored in the NIST/EPA/NIH mass spectral database (Version 2.0 g from May 19, 2011).

Rhipicephalus sanguineus collection

Engorged females of *R. sanguineus* were collected from naturally infested domestic dogs just after they started to drop off the host to ensure uniformity, this hosts had not received any acaricidal treatment for at least 45 days to avoid any negative interference, on many farms of the municipality of Ain Maabed (34° 48′ 17″ N, 3° 07′ 46″ E), Djelfa, Algeria.

The ticks were stored in cooled plastic boxes (≈15°C) to reduce their activity and immediately transported to the laboratory then thoroughly washed with distilled water and dried in paper toweling. Species identification was made under a binocular magnifier according to keys and descriptions provided by WALKER et al. (2003).

Preparation of the toxicological test

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

Adult immersion test

The AIT was performed as described in the literature (DRUMMONDS et al. 1973, FAO 2004) with minor modifications. In the groups of fifteen engorged female ticks, each was individually weighed to obtain groups with similar weights (0.5±0.1 g). The different groups of ticks dipped in 10 ml of each concentration for five minutes. All tests were replicated three times. After exposure, the engorged females were removed, dried then placed in Petri dishes that were incubated for fifteen days at 27 ± 2 °C and 80% relative humidity. Ticks were confirmed dead based on signs of hemorrhagic skin lesions, cuticular darkness, and lack of Malpighian tube movement. After two weeks, the eggs were weighed and transferred to tubes then placed in the incubator under the same conditions for larval hatching.

The egg production index (EPI), the reduction in hatching (HR), the reduction in oviposition (RO), the reproduction efficiency index (REI), and the efficiency of the extract (EP) were calculated according to 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 hatching/engorged females weight) × 20,000 (DRUMMONDS et al. 1973)
EP (%) = [(REI control − REI treated)/REI control] × 100 (DRUMMONDS et al. 1973)

Larval immersion test (LIT)

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

A number of 100 larvae were immersed for five min in tubes container with 10 mL of different concentrations of *Rosmarinus officinalis* essential oil, the tubes were closed and shaken vigorously for some seconds and then gently for five min.

Then these larvae were transferred with a paintbrush to dry over a paper towel. Next, they were placed to on filter paper (8.5 × 7.5 cm) (Whatman No. 1) that was folded and closed with clips forming a packet. The packets were incubated at 27–28 °C and ≥ 80% relative humidity.

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

Statistical analysis

The mortality values obtained from the various concentrations were considered as means. These results were subjected to a probit analysis to calculate the lethal concentrations and lethal times (LC50% LC90%, LT50%, and LT90%). This analysis was performed using the IBM SPSS Statistics program23 in Windows.

RESULTS

Yield and chemical composition of *Rosmarinus officinalis* essential oils

The oil yield of *Rosmarinus officinalis* obtained was 1.49%. The chemical composition by GC-MS (Table 1) revealed 50 compounds with a total percentage of 100%. Five major components have been 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 effect of *Rosmarinus officinalis* essential oil:

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

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

Also, it obtained a high proportion of inhibition of egg hatching by this essential oil of 60% of egg hatching for the maximum concentration, however, the newly hatched larvae did not survive and died a few hours after hatching (Table 2).

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

No.	RT	Compound name	Abundance %
1	7.27	α-Pinene	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	Benzenemethanol, α,α,4-trimethyl-	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)-, cis-	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	alfa.-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

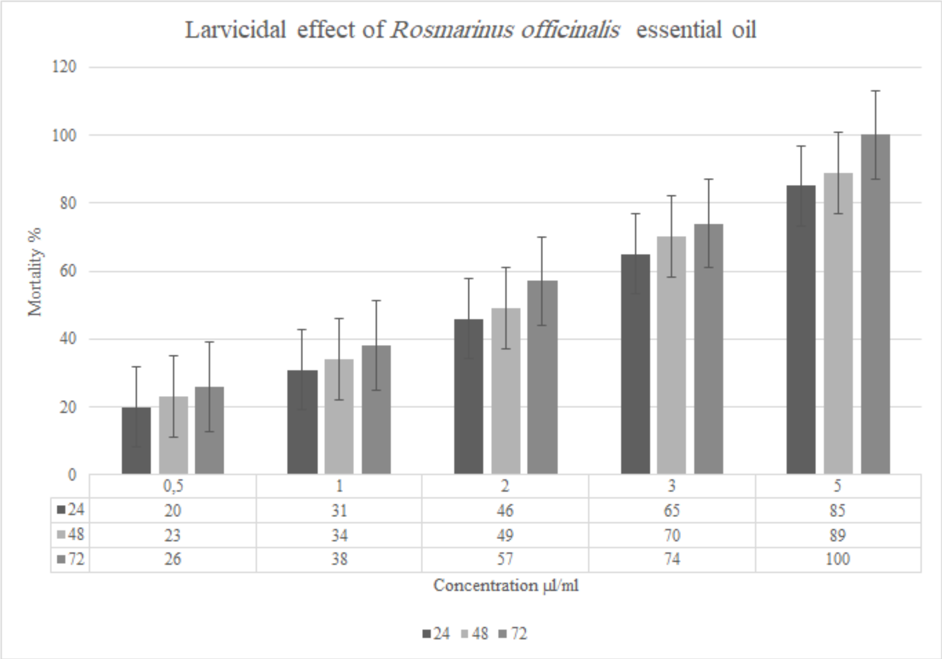

Figure 2. Chromatographic profile of *Rosmarinus officinalis* essential oil analyzed by CG-SM.

Table 2. Effect of Rosmarinus officinalis essential oil on the reproductive aspects of *R.sanguineus* females.

Concentration µl/ml	EPI (%)	RO (%)	REI	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, *Rosmarinus officinalis* essential oil showed a larvicidal effect against the larvae of *R. sanguineus* with a mortality rate that varies between 20% after 24h for the lowest concentration (0.5 µl/ml), up to 100% after 72 h when the larvae were exposed to the highest concentration (5 µl/ml) (Figure 3). this efficiency increased as both the time of exposure and concentration of oil increased, even more, 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 concentration of the essential oil.

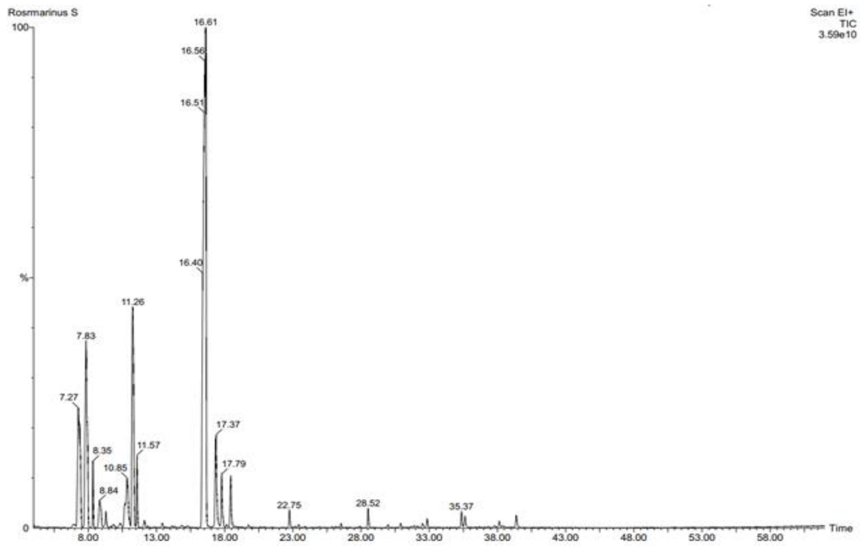

Figure 3. Evolution of mortality rate % in the *R.sanguineus* larvae treated with the different doses of *Rosmarinus officinalis* essential oil

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

A

Time (hours)	24	48	72
Regression line	Y = -0.95+0.41x	Y = -0.88+0.43x	Y =-0.85+0.51x
LC 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

*Calcule was not possible.

After 24h, a concentration of 2.286 µl/ml ensures the mortality of 50% of the larval stage, in addition, to ensure a 90% mortality the concentration of *Rosmarinus officinalis* must be equal to 5.380 µl/ml. After 48 h and 72 h of treatment, the calculations show respectively that the LC50% is 2.021 µl/ml and 1.635 µl/ml while the LC90% is 4.935 µl/ml and 3.783 µl/ml (Table 3).

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

DISCUSSION

The yield of *Rosmarinus officinalis* essential oil and its chemical characterization

The essential oil yield of *Rosmarinus officinalis* was 1.49%, it is higher than many other works done, noting that the yield of that collected in Kenya was 0.59% (MWITHIGA et al. 2022), in Portugal was 0.3–0.7% (SERRANO et al. 2002), also in Turkey was 0.71–0.94% (GURBUZ et al. 2016). However, it is lower if compared to those collected in Algeria, the yield of essential oil in Tbessa was 1.85- 2.29% (BOUTABIA et al. 2016). Regarding the chemical composition of this oil, it differs from those obtained by BAKKALI et al. (2018) in Morocco, in which 17 compounds account for around 75.6% of the total. The main constituents are: α pinene (32.64%), β humulene (8.71%), and Camphene (5.95%).

Also for the Indian rosmarinus essential oil the most important constituents were alpha -pinene (31.91%) and 1, 8 - cineole (14.66%). However, in France, KALOUSTIAN et al. (2002) recorded a camphor chemotype with a high level (30-45%). In addition, in Algeria, BOUTABIA et al. (2016) showed that 1,8-cineole is the predominant chemotype of *Rosmarinus officinalis* essential oil. But those of LOGRADA et al. (2013) noted that the chemical composition of essential oils of rosemary collected from 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 endo- and ectoparasites of livestock including ticks (MONDAL et al. 2013), the first intensive trials on the acaricidal activity were launched by KHAIDAROV (1971) evaluating 84 plants, currently at the global level 200 plant species have been registered for their repellent or acaricidal properties (ADENUBI et al. 2016). The orientation towards biocides is due to the abundance of plant secondary metabolites with toxicological activity, their low cost, and relatively lower toxicity to the environment and the 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) make plant extracts a better alternative to control tick populations (OLIVEIRA et al. 2016).

Toxicological tests of the present study reveal a considerable and variable sensitivity of *R. sanguineus* using *Rosmarinus officinalis* essential oil, translated by a significant reduction of the egg mass of the engorged females from 33.82% to 16.41% with a significant reduction of the reproductive efficiency index compared to the control group. Moreover, the essential oil was also toxic to larvae expressed by rates of low to very high mortality, which correlates with the extension of time from one concentration to the other, with an LC50 of 2.286 µl/ml for 24 hours, 2.021 µl/ml for 48 hours, and 1.635 µl/ml for 72 hours.

Compared to the tick species chosen for this work, DAEMON et al. (2009) and MONTEIRO et al. (2009) showed the efficacy of thymol on *R. sanguineus* larvae and pupae by a mortality rate that reached 100% at the concentration of 2 and 0.5%, respectively, However, in the case of non-engorged *R. sanguineus* larvae, only 37.7% mortality was recorded at the 2% concentration of thymol (DAEMON et al. 2009), besides GODARA et al. (2013) showed the in vitro efficacy of chloroform extract obtained from *Artemisia absinthium* on adults, eggs and larvae using the adult immersion test (AIT) which cause a mortality rate reached 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 package test (LPT) provokes 100% mortality of larvae with LC50 and LC95 values of 1.11% and 2.37%.

In Algeria, few works have been carried out on the fight against ticks by plant extracts, these 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 by 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 by adult immersion test (AIT) and larval packet test (LPT).

Noting that the toxicity of different extracts of some plants is not only limited to mortality, but it 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 ovaries (KONIG et al. 2020), salivary glands (REMEDIO et al. 2016), the synganglion (ROMA et al. 2013).

However, the 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, the duration of the test, the presence or absence of index host, the species and stage of ticks, also the plant, the extraction, type and the solvent, have made it difficult to compare studies and select the best plant species.

CONCLUSION

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

These results open 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 required to validate this control strategy to standardize an experimental control design by establishing correct doses to be administered to animals and determining side effects related to phytotoxicity.

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