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Chemical Composition of the Essential Oil from *Ruta chalepensis* L. Growing Wild and its Acaricidal Activity Against the Cattle Tick *Rhipicephalus annulatus* (Acari: Ixodidae)

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IN addition to a phytochemical screening of the essential oil of *Ruta chalpensis* aerial parts, this study aimed to discover its eco-friendly acaricidal activity against the cattle tick *Rhipicephalus annulatus* (Acari: Ixodidae). The essential oil obtained by hydro-distillation from fresh *R. chalepensis* aerial parts was analyzed by gas chromatography-mass spectrometry. Acaricidal activity was evaluated against *Rhipicephalus annulatus* ticks by using an adult immersion test. A total of 33 compounds were identified that represented 99.58% of the oil composition. The main constituents of the oils were 2-Nonanone (27.76%), 2-Undecanone (27.12%), 2-Heptyl acetate (10.79%), 2-Acetoxytridecane (4.24%), 5,6-diethenyl-1-methyl-Cyclohexene (3.31%), Davanone (2.87%) and 2-Decanone (2.41%). The results revealed the presence of a high percentage of the two main classes ketones (64.33%) and Esters (19.93%) with a low quantity of various terpenoid classes. The essential oil revealed strong acaricidal activity against semi-engorged females of *R. annulatus* ticks recording LC_{50}: 1.9% and LC_{90}: 10.3% on the 1st day post-treatment.

Keywords: *Ruta chalepensis,* Gas chromatography, Chemical composition, *Rhipicephalus annulatus*, *In vitro* Acaricidal activity.

Introduction

Rutaceae is among the main families investigated for their phytochemical and pharmacological properties. Rutaceae includes around 160 genera, and it is commonly known as the citrus family [1]. The *Ruta* plant (Sadab) has evergreen sub-shrubs 20–60 cm tall with strong odor and is distributed in temperate and tropical countries.

Ruta chalepensis is a Mediterranean plant, native to dry, sunny environments, and has been used for medicinal purposes since ancient times as an antirheumatic, an antispasmodic, an aphrodisiac, and a treatment for snakebites, headaches, and wounds [2,3,4]. Pharmacological evaluations of *R. chalepensis* established its effects as an analgesic, anthelmintic [5], anticancer [6,7], antiacetylcholinesterase [8], anti-inflammatory [5], anticonvulsant and sedative [9], antimicrobial, antioxidant [10,11], antiparasitic, menstrual disorders [12], abortive and antirheumatic activity [13], it is also useful in perfumery [14]. *Ruta chalepensis* is a rich source of phenolics, flavonoids [10,15], terpenes, furanocoumarins, and alkaloids [16,17,18]. Coumarins isolated from *R. chalepensis* exhibited an antifertility effect [19]. Furthermore, (El Sayed *et al.* [20] found that quinoline alkaloids isolated from this plant gave mutagenic, ganglionic-blocking, curare-like, and spasmolytic effects.

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The composition of *R. chalepensis* essential oil contained 0.19% volatile compounds with over 90% ketones, principally methyl nonyl ketone with some methyl heptyl ketone [18]. The major constituents of the essential oil extracted from *R. chalepensis* aerial parts are 2-undecanone, 2-nonanone (, *β*-phellandrene, and 2-methyl-octyl acetate [21,22]. The distinctive odor of the plant or its essential oil is due to methyl n-nonyl ketone [23]. In Tunisia, the percentage of yield was about 7.8% by supercritical CO_2 extraction in comparison with about 5.5% obtained by hydrodistillation process [24].

Ruta plants are reported in many countries with different chemical constituents [25-31] by using the former sources tabulated the chemical constituents of *Ruta* oil into three groups based on the concentration of their essential oils in undecane-2-one. The sources in the first group have at least 60% Algeria, Argentina, Tunisia, and India are all included. While Italy, Turkey, and Iran are among the second group of countries that includes the range between 30 and 60%. The third group is Saudi Arabia, with content under 30% undecane-2-one. The author attributed this variation to many factors, including the environment and climatic conditions (temperature, humidity, soil, location, and sunshine).

Ruta chalepensis essential oil has insecticidal activity against stored products pests as *Tribolium confusum, Trogoderma granarium* and *Tribolium castaneum* [32-34] and mosquitoes as *Aedes albopictus*, *Anopheles quadrimaculatus*, *Culex pipiens* and *Aedes aegypti* [35-38]. Moreover, the essential oil revealed anthelmintic activities against *Haemonchus contortus* infecting sheep [39].

The chemical composition of the essential oil extracted from *R. chalepensis* in Saudi Arabia's Jizan region has never been determined. Furthermore, the acaricidal activity of its essential oil has never been tested. Therefore, this study was designed to determine the chemical composition of *R. chalepensis* essential oil and evaluate its acaricidal activity against semi-engorged females of the cattle tick *Rhipicephalus annulatus*.

Material and Methods

Plant materials

The pneumatic parts of *R. chalepensis* L. were assembled from Jabal Fayfa, Jazan region, Saudi Arabia in December 2018, Locally, *R. chalepensis* is known in Jizan as Shazab. The plant was identified by Dr. M. Remesh (Biology Department, Faculty of Science, Jazan University, Saudi Arabia). A voucher specimen (117/2018) has been deposited in the Herbarium (JAZUH), Jazan University.

The study area is in a subtropical dry zone with mild winters and hot summers [40]. The total annual rainfall from January 2000 to August 2020 was 1560 mm, with the majority of rain in July and August. In the warm months, the average minimum air temperature ranged from 22.9 to 31.0 °C, While the average maximum air temperature ranged from 30.6 to 38.3 °C as in most monthly summer. (Meteorological Station, 7 m atop sea level, Jizan City; 16°53′48.5″ N 42°35′02.4″ E).

Extraction of essential oil

The fresh aerial parts of *R. chalepensis* L. (100 g) were sliced into small fragments, then hydro-distilled for 3h utilizing a Clevenger device and repeated 3 times to obtain the percentage of oil. The volatile oil obtained was desiccated by sodium sulphate anhydrous and reposit at 4 ◦C until analysis.

Gas Chromatography-Mass Spectrometry

Gas chromatography with a flame ionisation detector (GC-FID) and mass spectrometry (GC-MS) were used to analyse the samples [41]. Quantitative analysis was performed using a Clarus 500 GC (Perkin-Elmer Inc. Wellesley, PA, USA) chromatograph kitted with a FID detector and capillary column ZB-5 (30 m 0.25 mm i.d. 0.25 m film thickness; Phenomenex Inc. Torrance, CA, USA).1 µL was the injection volume. The temperature of the GC oven was programmed to rise by 3°C / min from 50 to 250°C. The gas phase was helium (1.2 mL/ min^{-1}). Temperatures for the injector and detector have been set at 250°C. Using the software Total Chrom 6.2, the % composition of the Essential oil was estimated from the GC peak areas without any correction factors (Perkin-Elmer Inc., Wellesley. PA. USA).

A GC/MS apparatus (Shimadzu Corporation, model; QP2010 Ultra, Kyoto, Japan) was used for the analysis of gas chromatography/mass spectrometry. The constituents of the sample were differentiated using a 30 m long × 0.25 mm i.d. capillary column coated with a 0.25 µm film thickness stationary phase (Rtx-5MS, Restek Corporation, U.S). Helium (99.999%) was used as the carrier gas, with a constant Linear Velocity of 36.3 cm/sec. AOC-20i+s auto-injector was used to

inject µL L of the sample. In split less mode, the injector was set to 290.00 °C. The heat of the GC furnace was set as 5 minutes at 30 °C, followed by 4.5 minutes at 300 °C at 4 °C/min. The Ion Source temperature in the MS was set to 230.00 °C, and the Interface temperature was held to 280.00 °C. Total Ion Chromatogram (TIC) for m/z range 30-700 was generated. The GC peaks were identified by comparing their mass spectra to the National Institute of Standards and Technology database (NIST, Ver.11). Each component's relative percentage amount was calculated by comparing its peak area to the total area of peaks in the chromatogram.

The cattle tick *Rhipicephalus annulatus*

Semi-engorged females of *R. annulatus* ticks were collected from naturally infested cattle from a private farm in Kafr Elsheikh and examined using a stereomicroscope according to the key of Walker *et al.* [42]. The ticks were immersed in distilled water rapidly for cleaning and then dried using filter paper.

Acaricidal activity of the essential oil

Adult immersion test was used in the bioassay of *R. chalepensis* essential oil (REO) against *R. annulatus* ticks according to Abdel‐Ghany *et al.* [43]. Five different concentrations (10, 5, 2.5, 1.25, and 0.625%) of REO were prepared in 70% ethyl alcohol. These concentrations were chosen based on a pilot test. Three replicates (10 ticks/replicate) were used for each concentration. Thirty semi-engorged adult ticks were immersed in 5 ml of each concentration for 2 min. After immersion, ticks were dried using filter paper then put into plastic tubes and maintained at 25±1 °C 75–80% relative humidity. The control group was immersed in 5 ml of 70% ethyl alcohol for 2 min. Examination of ticks was performed after 24 and 48 h where ticks that exhibited cuticle with dark color and were unable to move were considered dead.

Statistical analysis

The mortality percentages of ticks were analyzed statistically by F test followed by Duncan test using SPSS program version 20. LC_{50} and LC_{90} values were calculated by applying regression equation analysis to the probit-transformed data of mortality. The dose-response data were analyzed by the probit method [44] using Ehab software.

Results and Discussion

Chemical composition of the oil

Ruta chalepensis fresh aerial parts give a bluish essential oil with a characteristic odor. The chemical composition of *R. chalepensis* essential oil is summarized in (Table 1, Fig. 1-2). Thirty-four compounds were identified representing 99.58% of the oil composition. The main constituents were 2-Nonanone (27.76), 2-Undecanone (27.12), 2-Heptyl acetate (10.79%), 2-Acetoxytridecane (4.24%), 5,6-diethenyl-1-methyl-Cyclohexene (3.31%), Davanone (2.87%) and 2-Decanone (2.41%). Chemical composition was characterized by the low prevalence of undecan-2-one. The bluish color observed in *R. chalepensis* essential oil may be returned to the first detection of chamazulene (0.58%) which has not been reported in *R. chalepensis* essential oil before. This can be attributed to that the plant was collected from a mountainous region at the equator of a particularly meteorological nature.

The yield is 0.45% which was found to contain aldehydes and ketones (64.33%), esters (19.93%), monoterpene hydrocarbons (1.58%), oxygenated monoterpenoids (2.07%), oxygenated sesquiterpenoids (4.13%) and oxygenated diterpenoids (1.17%). Our results were in line with Mejri *et al.* [31] who reported that the chemical constituents of the essential oil of *Ruta* were low in content from undecane-2-one grown in Saudi Arabia with a percentage less than 30%. Mejri *et al.* [24] stated that the chemical composition of essential oil differs from one year to another. The characteristic odor of the oil is due to the presence of 2-Undecanone [23]. When taking into account ontogenetic or environmental variability factors, the distinction between chemotypes based on quantitative differences between metabolically linked chemicals may not be sufficient.

TABLE 1. Chemical compositions of the essential oil from the aerial parts of *Ruta chalepensis*.

	RT	Name	Area (%)
1	2.618	Unknown	0.42
2	10.921	(E)-2-Hexenal	0.51
3	17.104	2-Octanone	0.74
4	17.575	alpha-Phellandrene	0.98
5	18.599	D-Limonene	0.6
6	21.658	2-Nonanone	27.76
7	21.754	2-Nonanol	1.23
8	21.868	Nonanal	0.91
9	22.923	3,4-diethenyl-3-methyl-Cyclohexene	0.77
10	23.103	1-Methylheptyl acetate	0.82
11	23.236	5,6-diethenyl-1-methyl-Cyclohexene	3.31
12	23.353	(+)-Camphor	1.58
13	24.522	(-)-Terpinen-4-ol	0.49
14	25.118	2-Decanone	2.41
15	26.82	2-Heptyl acetate	10.79
16	27.402	2-Tridecanone	0.34
17	28.452	Bornyl acetate	0.35
18	29.04	2-Undecanone	27.12
19	29.318	n-Nonanyl acetate	0.65
20	30.052	Acetic acid, dec-2-yl ester	0.37
21	31.095	2-Pentadecanone	1.59
22	32.042	2-Dodecanone	0.93
23	33.28	2-Acetoxytridecane	4.27
24	35.209	2-Tetradecanone	1.32
25	36.923	Elemol	0.91
26	37.25	E-Nerolidol	0.35
27	38.041	Davanone	2.87
28	38.337	4-(1,3-Benzodioxol-5-yl)-2-butanone	0.34
29	39.121	Apiol	0.48
30	42.115	Chamazulene	0.58
31	43.434	Pentadecane-2,4-dione	0.36
32	44.175	*o*-Anisic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester	1.84
33	49.643	*p*-Anisic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester	0.84
34	51.284	Phytol	1.17
		Oil yield	0.45%
		Total unidentified	0.42
		Aldehydes and Ketones	64.33
		Esters	19.93
		Monoterpene hydrocarbons	1.58
		Oxygenated monoterpenoids	2.07
		Oxygenated sesquiterpenoids	4.13
		Oxygenated diterpenoids	1.17
		Others	6.37
		Total Identified	99.58

RT = Retention time in seconds

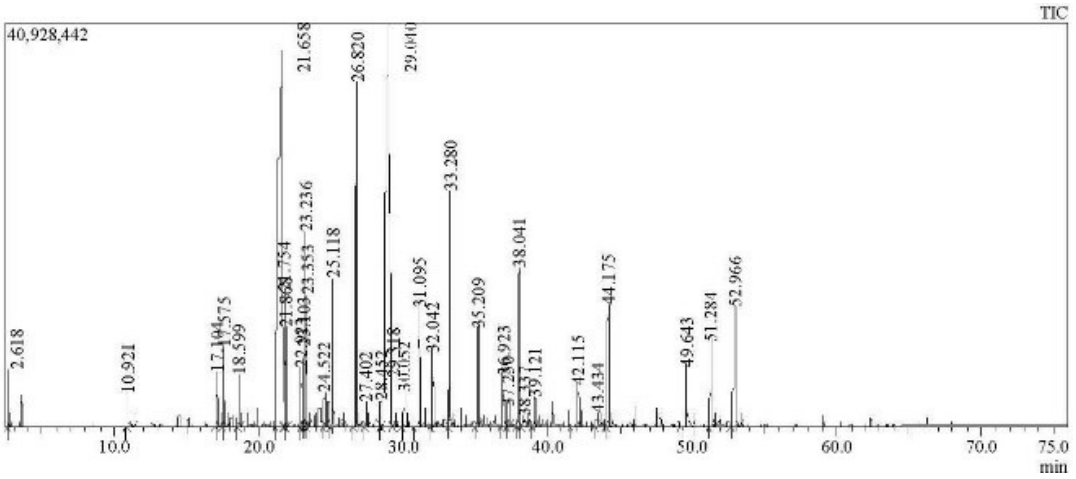

Fig. 1. Gas chromatogram of the essential oil from the aerial parts of *Ruta chalepensis*.

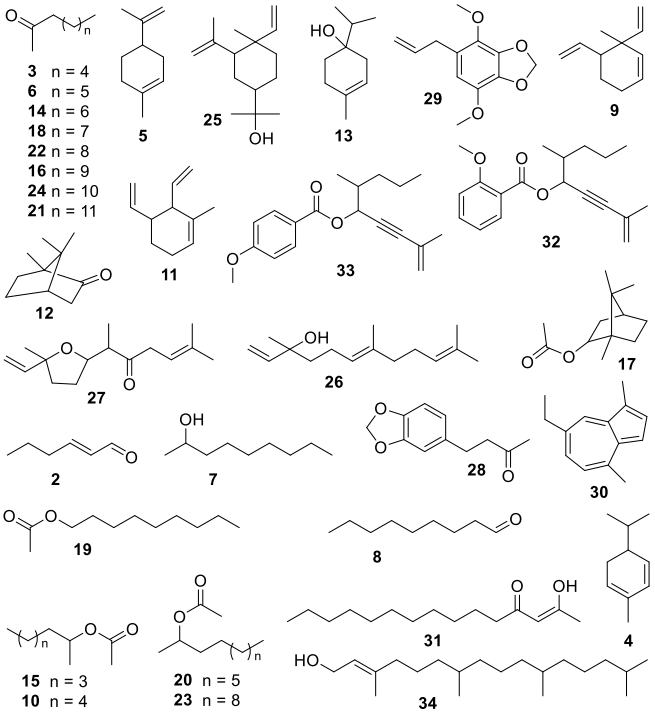

Fig. 2. The structure of the identified compounds from the aerial parts of *Ruta chalepensis*

In vitro acaricidal activity of the oil against ticks

The essential oil revealed strong acaricidal activity against *R. annulatus* ticks on the 1st day post-treatment without an increase in mortality on the 2nd day. The dead ticks seem to be darker in color and no movement with legs extending laterally in a straight form comparing with the live ticks in the control group. The mortality percentages of the ticks increased gradually to reach 100% at 10% of the essential oil. There are statistically significant differences between concentrations ($P<0.01$) in affecting tick mortality (Figure 3). The calculated LC_{50} and LC_{90} were 1.9% and 10.3%, respectively (Figure 4).

The toxicity of *Ruta chalepensis* essential oil against *R. annulatus* ticks can be attributed to its high

content of 2-undecanone, an aliphatic ketone that has been proven previously to possess insecticidal activity [34]. It can penetrate through natural orifices of the tick body such as spiracular plates and cause dysfunction of the respiratory system and death occurs rapidly during the first day post-treatment. In addition, the rapid action of essential oils on harmful insects is due to a neurotoxic effect, where it can penetrate through the cuticle and come into contact with the nerve endings of the trachea of the insect and then cause neurotoxic activity and rapid death [45].

The tick that succeeded in tolerating the oil could stay alive during the second day. Therefore, treatment of ticks with *R. chalepensis* essential oil is not time-dependent this was contrary with the finding of Najem *et al.* [34] who reported that this oil is time-dependent when tested against the stored products insect *Tribolium castaneum* (Coleoptera: Tenebrionidae) because this insect took the oil in its food and the extended effect may be due to the damage in the digestive system. Contrary to this study, the camel tick *Hyalomma dromedarii* revealed time-dependent in response to rosemary, garlic, neem, and cypress oils [46]. In general, this is the first evaluation of *R. chalepensis* essential oil as an acaricide against ticks. However, there are a few studies that evaluated the effect of *R. chalepensis* as an essential oil or extract against insects. Asiry and Zaitoun [33] showed that LC_{50} of *R. chalepensis* acetonic extract was 576 ppm after 2 days against the insect *Trogoderma granarium* everts (Coleoptera: Dermestidae). Pérez López *et al.* [38] stated that *R. chalepensis* essential oil showed larvicidal activity against the mosquito *Aedes aegypti* (Diptera: Culicidae). New Orleans and local population strains, producing LC_{50}: 2.69 and 20.13 g/mL, respectively at 24 h. Conti *et al.* [35] found that essential oils extracted from wild and cultivated *R. chalepensis* plants revealed larvicidal activity against mosquito *Aedes albopictus* recording LC_{50}: 35.66 and 33.18 ppm, respectively with mortality dosage dependent in both oils.

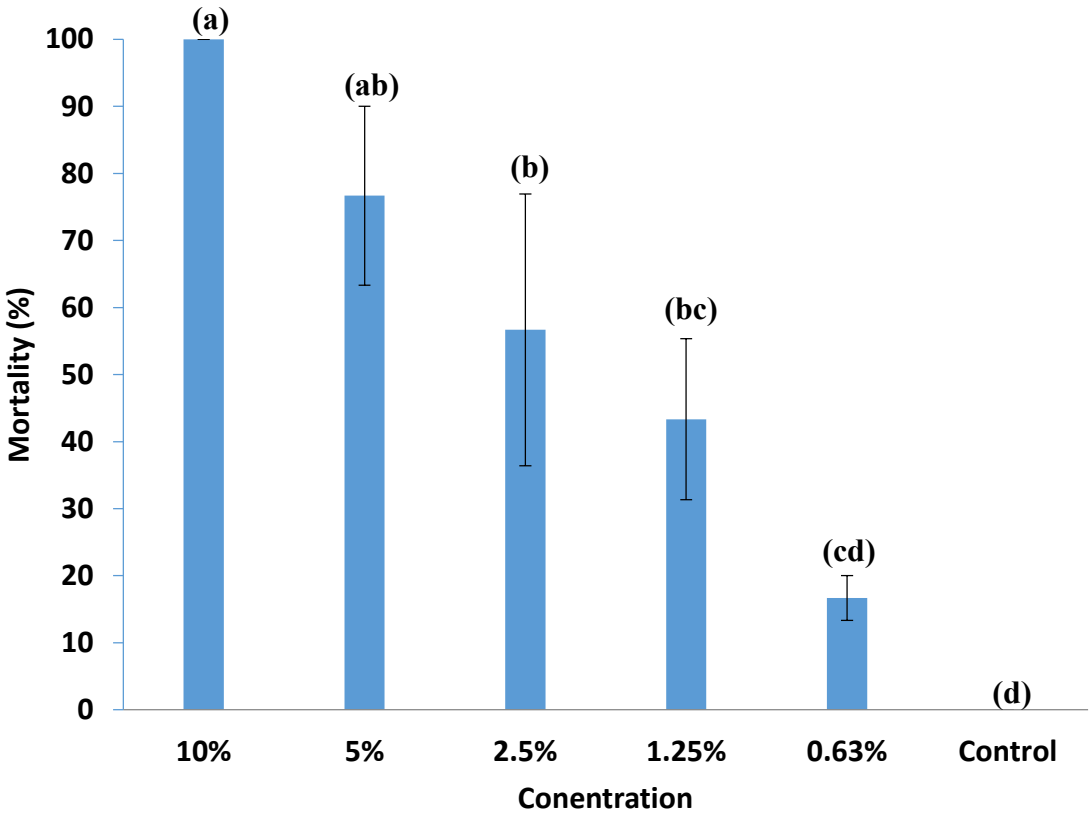

Fig. 3. Mortality percentages of semi-engorged females of the cattle tick *Rhipicephalus annulatus* treated with different concentrations of *Ruta chalepensis* essential oil. Small letters indicate significant differences between concentrations affecting tick mortality at P<0.01.

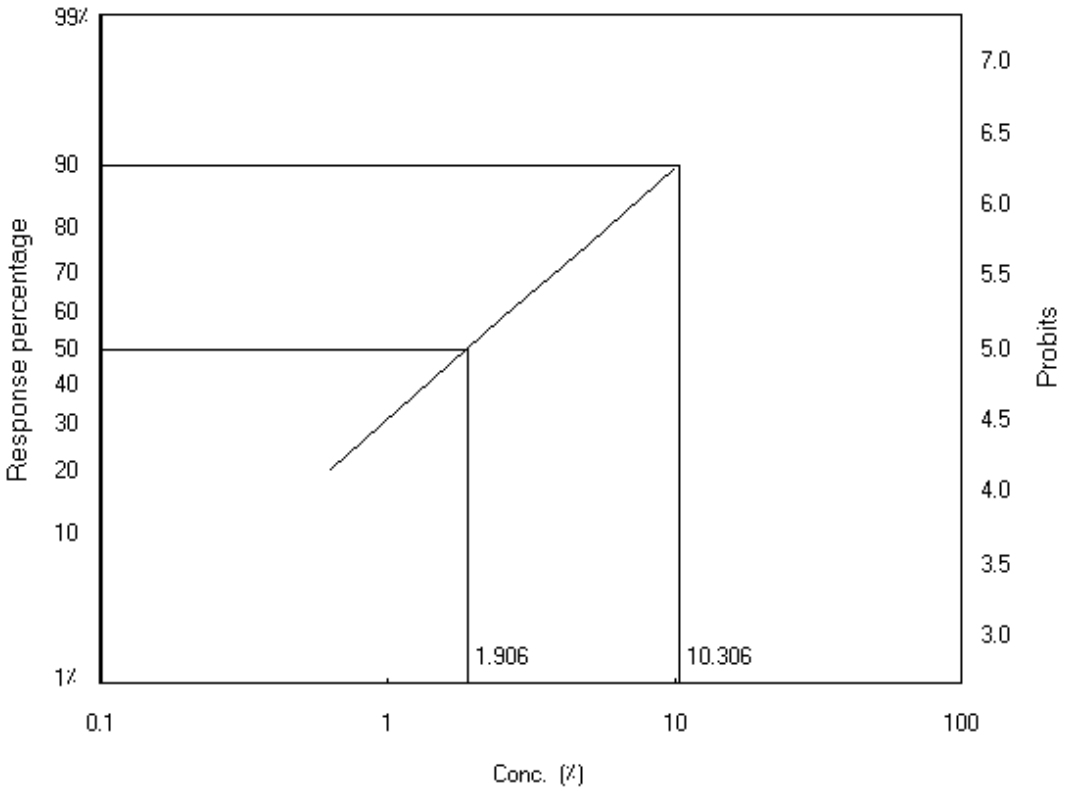

Fig. 4. Toxicity line of semi-engorged females of the cattle tick *Rhipicephalus annulatus* treated with *Ruta chalepensis* essential oil showing LC_{50} (1.9%) and LC_{90}= 10.3%.

Conclusion

This study suggests a wide variation in the essential oil composition of *Ruta chalepensis* through various places in the same country (Jizan and Makkah -Saudi Arabia) or different countries, which may illustrate a new diversity in the quality and biological activity of this oil. The essential oil revealed strong acaricidal activity against *R. annulatus* ticks on the 1st day post-treatment without an increase in mortality on the 2nd day. The calculated LC_{50} and LC_{90} were 1.9% and 10.3%, respectively.

Author contribution

All authors shared the design of the study. AAY and AAMA participated in preparing and analyzing essential oil. HSMA and SA evaluated the acaricidal activity of the essential oil against the cattle tick. AAY, HSMA, and SA wrote the draft of the manuscript. The final version of the manuscript was revised and agreed for publication by all authors.

Ethical approval

This study was approved by the Ethical Committee for Medical and Veterinary Research at the National Research Centre (NRC), Egypt in accordance with local laws and regulations. (Approval protocol No. 1783022023).

Disclosure statement

No potential conflict of interest was reported by the authors.

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التركيب الكيميائي للزيت العطري لنبات *روتا كالليبنسيس* النامي بريا ونشاطه القاتل ضد قراد الأبقار *ريبيسيفالس أنيولاتس* (أكاري: إكسوديدي)

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بالإضافة إلى الفحص الكيميائي النباتي للزيت العطري للأجزاء الهوائية من نبات *روتا كالليبنسيس* هدفت هذه الدراسة إلى اكتشاف نشاطه كمبيد صديق للبيئة ضد القراد. تم تحليل الزيت العطري الذي تم الحصول عليه عن طريق التقطير المائي من الأجزاء الهوائية الطازجة لنبات *روتا كالليبنسيس* بواسطة تحليل كروماتوغرافي الغاز ومطياف الكتلة. تم تقييم نشاطه كمبيد ضد القراد *ريبيسيفالس أنيولاتس* باستخدام اختبار الغمر للأطور الكاملة. تم تحديد 33 مركباً تمثل 99.58% من تركيبة الزيت. المكونات الرئيسية للزيوت هي 2-نونانون (27.76%)، 2-أنديكانون (27.12%)، 2-هيبتيل أسيتات (10.79%)، 2-أسيتوكسيتريدكان (4.24%)، 6،5-ثنائي إيثيل-1-ميثيل-سيكلوهكسين (3.31%)، دافانون (2.87%)، 2-ديكانون (2.41%). أظهرت النتائج وجود نسبة عالية من الصنفين الرئيسيين الكيتونات (64.33%) والاسترات (19.93%) مع كمية منخفضة من مختلف أصناف التربينويد. كشف الزيت العطري عن نشاطه كمبيد قوي ضد الإناث نصف متغذية لقراد *ريبيسيفالس أنيولاتس* مسجلاً 1.9% كتركيز مميت لنصف عدد الأفراد و 10.3% كتركيز مميت لـ 90% من الأفراد المعاملة في اليوم الأول بعد العلاج.

الكلمات الدالة: روتا كاليبنسيس ، كروماتوغرافيا الغاز، التركيب الكيميائي ، ريبيسيفالس أنيولاتس ، النشاط القاتل في المعمل.