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Acaricidal and Repellence of *R. appendiculatus*, and GC-MS Chemical Content of Essential Oils from Three South African Ethno-Veterinary Plants

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The advancement of the livestock farming in sub-Saharan Africa is restricted by tick infestation. With conventional pesticides posing a threat to human and the environment, natural products are alternative anti-tick source. This study characterized the essential oils from *Tithonia diversifolia, Lavandula angustifolia* and *Cymbopogon citratus* leaves by GC-MS and, for acaricidal and repellence of *R. appendiculatus*. GC-MS analysis detected various chemical compounds, some of the isolated compounds have anti-tick properties. The repellence of *T. diversifolia* essential oil at 5% v/v was the weakest against adult ticks. All essential oils at 15% v/v paralysed nymph after 20 mins, with *C. citratus* after 24 h causing high mortality of nymph and adult ticks. Exposure to *L. angustifolia* did not achieve 50% mortality after 24 h. All essential oils caused complete inhibition of moulting of engorged larvae. The study demonstrated that the essential oils of the ethno-veterinary plants may be a source of anti-ticks agents.

Keywords: *Rhipicephalus appendiculatus*, *Tithonia diversifolia*, *Lavandula angustifolia*, *Cymbopogon citratus*, Acaricidal, Repellence.

INTRODUCTION

Livestock plays a vital role in the socio-economic activities of the people, especially in developing countries where they provide food security and income [1]. In 2008, National Department of Agriculture estimated over two-third of the 14.1 million cattles in South Africa belonged to communal farmers indicating the livestock production is important to sustaining the livelihoods of many households [2,3]. Ticks inflict a great burden on livestock productivity as they decrease fertility, trigger skin irritation and suck blood, ultimately leading to death [4]. The conventional pesticides used to control ticks are expensive to resource poor farmers and some ticks are developing resistance against these drugs in addition to posing a threat to the environment [5]. Thus, many farmers have resorted to alternative measures which include the use of medicinal plants to treat and control livestock parasites [5,6]. It is, therefore, necessary to scientifically investigate and validate these plants as a more ecologically safer alternative control measure.

Ticks are important vector that can transmit a large variety of microorganism such as protozoa, bacteria (specifically rickettsiae and spirochaetes), viruses and even helminths and cause conditions like paralysis, toxicosis, irritation and allergy [7,8]. They are in the order Acari and consist of two main families: Argasidae and Ixodidae. Argasids have a soft and flexible cuticle on the dorsal surface of their bodies, while ixodids have a hard and sclerotized dorsal plate [9]. Ixodids have four powerful pairs of legs equipped for active running with protruding mouthparts, while argasids have legs adapted for crawling with mouthparts of located on the underside of the tick [10]. Ticks detect their hosts with the sensory receptor located on the front legs and mechanical stimuli induce them to bite [11]. Hard ticks are more parasitic than soft ticks [10,12] because they inflict painless bites that go unnoticed for a long time [13] and can feed for extended periods of time varying from several days to weeks [14]. An example is *Rhipicephalus appendiculatus*, also known as the brown ear tick, a hard tick found in Africa, where it spreads the parasite *Theileria parva*, the cause of East Coast fever in cattle [15].

Various plant products, crude extracts and essential oils have been evaluated for their repellent and acaricidal properties

against all the stages (adult, nymph, larva and egg) of economically important tick species with encouraging results [16-18]. Among them, essential oils have been found to exhibit strong repellence activity against ticks [19]. Although studies have shown that susceptibility to a repellent varies between tick species and life stages, the molecular basis for these differences is still unknown [20]. Essential oils are complex mixtures of volatiles organic secondary metabolites of plants and they are constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds [21]. Essential oils are concentrated essences responsible for the characteristic the fragrance of plants [22]. The composition of essential oils varies considerably between aromatic plant species and varieties as well as within the same variety from different geographical areas [23]. Variation has also been reported in the chemical composition of essential oils from same plant at different growing periods [24]. Essential oils have been known for the various properties they possess for many centuries, including to protect the plants from bacterial, fungal or viral attacks [25] as well as to attract the insects which cause dispersal of the pollens [26,27]. The objective of the study was to evaluate the essential oils of three selected ethno-veterinary plants from Ermelo community Mpumalanga province of South Africa (*Tithonia diversifolia*, *Lavandula angustifolia* and *Cymbopogon citratus*) for their chemical profiles as well as to evaluate the repellence and acaricidal properties against the tick (*Rhipicephalus appendiculatus*).

EXPERIMENTAL

Collection of plants: Three plants *viz. Tithonia diversifolia* (Mexican sunflower), *Lavandula angustifolia* (lavender) and *Cymbopogon citratus* (citronella grass) were collected from Nooitgetdacht Agriculture Development Centre, Ermelo (GPS coordinates 26.5º31′59.99″ S and 29.9º58′59.99″ E), Mpumalanga Province, South Africa. Herbarium specimens of each plant sample were mounted and sent to the SANBI for proper identification.

Extraction of essentials using a hydrodistiller: The essential oil of leaves of plants was extracted by hydrodistillation methods with the Clevenger apparatus. The fresh leaves (100 g) of the plants in distilled water (200 mL) were heated to breakdown the plant cell structure, which freed the essential oil. Essential oil molecules were carried along a pipe and channelled through a cooling tank, where they returned to their liquid state (still water and oil) and were collected through a process of condensation. Since the density of oil was lighter than water, it was easy to separate the essential oil from the water by using a simple siphoning method.

Gas chromatography-mass spectrometry (GC-MS) analysis: The major chemical constituents of the essential oils was obtained using gas chromatograph (Shimadzu QP-2010) coupled with an electron impact quadruple mass spectrometer detector (Japan). The electron ionisation energy was 70 eV, scan range of 40-400 μ and scan speed of 1250 μ/s at an interval of 0.30 s. The GC was equipped with a DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm film thickness Agilent, USA). The carrier gas was helium at 1.3 mL/min flow rate and at 41.6 cm/s linear velocity ($\bar{u}$). The initial oven temperature of 60 ºC and held for 10 min, was programmed to increase to 210 ºC at a rate of 3 ºC/min and held for 10 min at 210 ºC. A 1% (v/v) solution of each essential oil sample in hexane was prepared and 1 μL was injected using a 1/40 split ratio. A homologous series of C9-C24 alkanes was used to determine the GC retention indexes (RI) of the oil constituents. The identity of compounds was resolved by comparing the RI and mass spectral fragmentation with data from a commercial mass spectral database (Wiley, USA) and from previous studies [28].

Tick collection: The ticks were purchased from ARC-LNR Onderstepoort Veterinary Research and verified at the Department of Veterinary Science at the University of Pretoria, Onderstepoort campus. Pathogen free tick species used in this study was *Rhipicephalus appendiculatus.*

Tick repellence bioassay: The tick climbing repellence bioassay was adapted from a previous method [6]. In this assay, ticks were placed on dry platform surrounded by water in a beaker. In the middle of the platform was fitted a glass rod of approximately 23 cm in length and covered from the top with filter paper to about 4.5 cm. The filter paper was treated separately with 200 μL essential oils (5, 10 and 15% v/v) in acetone or acetone alone being the solvent of dissolution (solvent control). The blank was an untreated filter paper. Prior to the assay, 15 min was allowed for the acetone of the treatment and control filter paper on the glass rods to evaporate and also for the ticks to acclimatise. Thereafter, ten unsexed *Rhipicephalus appendiculatus* (3-5 weeks old) placed on the platform of the treated, solvent control and blank groups were observed initially at every 10 min for 60 min, and then at every 30 min for 120 min. Where ticks climbed onto the treated, solvent control or blank filter papers they were not repelled otherwise the ticks were repelled. The assay was done in five replicates for each concentration (5, 10 and 15% v/v) of the essential oils. Percentage repellence (R) was estimated from the equation:

$$R = \frac{(N_c - N_t)}{(N_c + N_t)} \times 100$$

where N_c and N_t are the numbers of ticks above the filter paper on the control and treated glass tubes, respectively.

Toxicity bioassay: The acaricidal effect of essential oils on nymph or adults ticks of *R. appendiculatus* (groups of 10) was estimated from the open filter paper method as recommended by WHO [29]. This assay was based on contact toxicity of ticks on treated filter papers. Different concentrations of each essential oil were prepared in acetone (5, 10 and 15% v/v) and corresponding concentrations for acetone alone, being the solvent of dissolution (solvent control). Then 200 μL of each essential oil or control solvent pipetted onto the surface of filter paper placed inside plastic cups was left for 10 min for solvent to evaporate. Thereafter, 10 nymphs per unsexed adult ticks were deposited into each plastic cup covered with meshed cloth. Upon sealing around the rim, the cups were stored in the dark at 20-21 ºC and 89-97% relative humidity. Mortality was monitored initially every 10 min for 4 h and later after 24, 48 and 72 h. Each treatment assay was done in three replicates and repeated thrice. Ticks were considered dead when no leg or antennal movements were observed.

Moulting inhibition bioassay: The moulting inhibition bioassay of essential oils was conducted by reported method [30]. In this assay, 50 µL of each diluted essential oil (5, 10 and 15% v/v) in acetone was infused onto filter paper. After 15 min for solvent evaporation, the filter paper was inserted into an appropriate glass vessel containing 50 engorged larvae of *R. appendiculatus* restricted by a mesh to avoid direct contact with the essential oil. The solvent control (acetone) and untreated filter paper was also assessed. The top of all glass vessels were plugged with cotton wool. The glass vessels with the engorged larvae were incubated at 28 ± 1 ºC and 85-97% relative humidity for eighteen days. The number of ticks that completely moulted 18th day after treatment was recorded.

Tick repellence bioassay: The effective concentration to repel 50%, 75% and 95% ($EC_{50,}$ EC_{75} and EC_{95}) of the ticks was calculated using probit analysis, a free software package (http://www.epa.gov/nerleerd/nerleerd/stat2.htm). The repellent effect was calculated as percentage repellence [31] according to the formula:

$$\text{Repellence } (\%) = 100 - \frac{\text{Mean no. of ticks on test}}{\text{Mean no. of ticks on control}} \times 100$$

where significance was found, Student's t-test *post-hoc* test resolved the differences between the repellence effects of essential oils.

Toxicity bioassay: Time dependent response graphs were generated from the plot of percentage mortality *versus* period of treatment for each concentration. The LC_{50} and LC_{95} were deduced from the graphs [32]. The shared variance (R^2) was determined for possible explanation for the influence of the essential oils of the different plants. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Due to the growing demand for safer and residue-free food products, there has arisen a significant opportunity for the development from natural sources of pesticides for tick control especially plant based pesticides [33]. This result from many problems associated with synthetic pesticides contamination of meat and milk in addition to tick resistance and relevant toxic effects on non-target species [34]. A group of plant secondary metabolites known as terpenoids are involved in defense against herbivorous arthropods and pathogens [35].

Extraction of essential oils: Various extraction methods have been used for removing of essential oils from plants parts with solvent and distillation extraction techniques being the most frequently used despite the associated limitation [36,37]. In this study, the Clevenger hydrodistillation technique was used to minimize the loss or denaturation of some chemical functional group especially the esters and monterpenes [36,37]. Various researchers used different distillation times, ranging from less than 60 min, to 180 min or 240 min [38]. This study demonstrated that with respect to oil yield, there are no advantages from increasing distillation time beyond 40 min [39].

The percentage of essential oil released based on fresh leaf weight after 3 h is presented in Table-1. The *L. angustifolia* (0.82% w/v) showed the highest yield followed by *C. citratus* (0.54% w/v) then *T. diversifolia* (0.38%). The extraction temperature was equal to the boiling of the water at atmospheric pressure (100 ºC). To reach this temperature and to obtain the distillation of the first droplet of essential oil, it was necessary to heat for 3, 5 and 3 min for *C. citratus, T. diversifolia* and *L. angustifolia*, respectively.

GC-MS: The compounds previously shown to possess anti-tick properties were also identified. A total of 37 chemical components were identified in the essential oil of the leaves

TABLE-1
EXTRACTION YIELD OF *C. citratus, T. diversifolia* AND *L. angustifolia* ESSENTIAL OILS

Plant sample	Minimum (%)	Maximum (%)	Range (%)	Mean (%)	Standard deviation
C. citratus	0.25	0.67	0.42	0.54	0.22
T. diversifolia	0.18	0.38	0.20	0.38	0.12
L. angustifolia	0.54	0.92	0.38	0.82	0.19

TABLE-2
CHEMICAL COMPOSITION OF *Cymbopogon citrates*

No.	Name of compound	m.f.	m.w.	Retention times	Peak areas (%)
1	2-Thujene	$C_{10}H_{16}$	136	3.88	8.05
2	Linalool	$C_{10}H_{18}O$	154	4.90	0.96
3	Citral	$C_{10}H_{16}O$	152	6.68	44.7
4	2-Undecanone	$C_{11}H_{22}O$	170	9.55	2.23
5	Geranic acid	$C_{10}H_{16}O_2$	168	7.84	14.5
6	Citronellol	$C_{10}H_{20}O$	156	6.42	0.62
7	β-Pinene	$C_{10}H_{16}$	136	3.87	6.70
8	*p*-Cymene	$C_{10}H_{14}$	134	4.30	16.31
9	Trifluoroacetyl-lavandulol	$C_{12}H_{17}F_3O_2$	250	7.38	4.32
10	2-Tridecanone	$C_{13}O_{26}O$	198	9.55	2.23
11	2-Dodecanone	$C_{12}H_{24}O$	184	9.55	2.23
12	1,3,8-*p*-Menthatriene	$C_{10}H_{14}$	134	5.42	2.69
13	*p*-Cymenene	$C_{10}H_{12}$	134	4.92	5.51

14	1,3-Dibromo-pentane	$C_5H_{10}Br_2$	230	14.30	1.44
15	Tetratetracontane	$C_{44}H_{90}$	619	12.14	3.89
16	Pinacol	$C_6H_{14}O_2$	118	7.03	2.33
17	2-Nonadecanone	$C_{19}H_{38}O$	283	7.10	6.68
18	Octadecane	$C_{18}H_{38}$	254	12.45	3.89
19	Nonadecane	$C_{19}H_{40}$	268	13.16	3.61
20	*m*-Toluamide	C_8H_9NO	135	6.02	3.32
21	Geraniol	$C_{10}H_{18}O$	154	6.71	1.14
22	Heptadecane	$C_{17}H_{36}$	240	12.45	3.89
23	2-Methyl-5-(1-propenyl)pyrazine	$C_8H_{10}N_2$	134	13.93	4.09
24	1,3-Cyclopentadiene	C_5H_6	66	5.87	5.86
25	Acetophenone	$C_9H_{10}O$	134	6.02	3.32
26	4-Methyl-1-(1-methylethnyl) cyclohexene	$C_{10}H_{18}$	138	6.14	4.27
27	2-Isopropenyl-5-methylhex-4-enal dimethyl-6-oxo, [S-(*E*)]-	$C_{10}H_{16}O$	152	7.03	20.23
28	2-Methyl-benzoxazole	C_8H_7NO	133	11.77	2.26
29	Triacontane	$C_{30}H_{62}$	423	15.76	4.29
30	Nonacosane	$C_{29}H_{60}$	408	13.16	3.61
31	Tetracosane	$C_{24}H_{50}$	339	14.15	4.51
32	2,3-Epoxy-geranylacetate	$C_{12}H_{20}O_3$	212	7.20	1.19
33	2-Octanone	$C_8H_{16}O$	128	7.23	1.58
34	*trans*-α-Bergamotene	$C_{15}H_{24}$	204	9.51	0.51
35	Geranyl butanoate	$C_{14}H_{24}O_2$	224	13.16	0.87
36	1-(Hydroxymethyl)-4-(4-metho-xyphenyl)-10-oxa-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3, 5-dione	$C_{16}H_{15}NO_5$	301	14.12	0.78
37	Phytol	$C_{20}H_{40}O$	297	14.07	0.18

Key
- High relative amount compound with no anti-tick properties
- High relative amount compound with anti-tick properties
- Low relative amount compound with anti-tick properties

TABLE-3
CHEMICAL COMPOSITION OF *Tithonia diversifolia*

No.	Name of compound	m.f.	m.w.	Retention times	Peak areas (%)
1	Ethylene oxide	C_2H_4O	44	4.216	2.04
2	1-Butanamine, 3-methyl-	$C_5H_{13}N$	87	8.222	0.71
3	*N*-(3-Methylbutyl)acetamide	$C_7H_{15}NO$	129	8.532	3.11
4	Cyclopentane, 2-n-octyl-	$C_{13}H_{24}$	180	9.008	3.33
5	Cyclobutanol	C_4H_8O	72	9.056	0.73
6	dl-Phenylephrine	$C_9H_{13}NO_2$	167	9.206	1.53
7	Phenylephrine	$C_9H_{13}NO_2$	167	9.307	1.69
8	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	9.623	2.31
9	1,3-Cyclohexanediol	$C_6H_{12}O_2$	116	9.778	2.08
10	Amphetamine	$C_9H_{13}N$	135	9.869	1.69
11	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	9.933	17.53
12	8-[*N*-Aziridylethylamino]-2,6-dimethyloctene-2	$C_{14}H_{28}N_2$	225	10.12	0.85
13	Benzenemethanol, α-[(methylamino)methyl]-	$C_9H_{13}NO$	151	10.677	0.58
14	Folic acid	$C_{19}H_{19}N_7O_6$	441	10.746	1.32
15	Acetic acid, hydroxy [(1-oxo-2-propenyl)amino]-	$C_5H_7NO_4$	145	10.816	1.11
16	*cis*-Vaccenic acid	$C_{18}H_{34}O_2$	282	11.04	19.2
17	Octadecanoic acid	$C_{18}H_{36}O_2$	284	11.174	7.67
18	Acetic acid, [(aminocarbonyl)amino]oxo-	$C_3H_4N_2O_4$	134	11.944	0.85
19	*cis*-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310	19.336	4.5
20	4-Fluorohistamine	$C_5H_8FN_3$	129	19.593	2.20
21	2,3-Dimethoxyamphetamine	$C_{11}H_{17}NO_2$	195	12.479	0.74
22	Benzeneethanamine, 2-fluoro-β5-dihydroxy-*N*-methyl-	$C_9H_{12}FNO_2$	185	12.618	2.62
23	2-Propenamide, *N*-(1-cyclohexylethyl)-	$C_{11}H_{19}NO$	181	13.025	1.73
24	Erucic acid	$C_{22}H_{42}O_2$	338	13.271	5.82
25	Acetamide, 2,2,2-trichloro-	$C_2H_2Cl_3NO$	161	13.405	0.85
26	2-Methoxy-*N*-methylethylamine	$C_4H_{11}NO$	89	13.495	2.45
27	Acetic acid, chloro-, pentyl ester	$C_7H_{13}ClO_2$	164	13.565	4.97
28	*p*-Hydroxynorephedrine	$C_9H_{13}NO_2$	167	14.415	1.61
29	Metaraminol	$C_9H_{13}NO_2$	167	14.464	1.28

Key
- High relative amount compound with no anti-tick properties
- High relative amount compound with anti-tick properties
- Low relative amount compound with anti-tick properties

of *C. citratus* by GC-MS analysis (Table-2). The chemical compositions profile revealed that the most abundant compounds which are citral and [S-(*E*)]-dimethyl-6-oxo-2-isopropenyl-5-methylhex-4-enal, may possess anti-tick properties. While *p*-cymene and geranic acid also detected in relatively high amount may not have anti-tick activity. For the essential oil of *T. diversifolia*, a total of 29 compounds were identified (Table-3). The relatively high amount of *n*-hexadecanoic acid may be attributed to anti-tick activities of *T. diversifolia* while *cis*-vaccenic acid may not be associated with the anti-tick activities. However, the relatively low amount of octadecanoic acid may also be attributed for anti-tick activity of the plant. Thirty-two chemical components were identified in the leaf essential oil of *L. angustifolia* (Table-4). The major components of the oil found were linalyl acetate and linalool in decreasing order which have both been attributed for anti-ticks properties of *L. angustifolia* [40]. The relatively low amount of α-terpineol and *trans*-caryophyllene may also be responsible for anti-tick activity of the plant [41]. Jaenson *et al*. [42] demonstrated that the 100% repellence of *L. angustifolia* essential oil to *Ixodes ricinus* nymphs when diluted to 30% in 1,2-prop-anediol. This study has shown that this essential oil has broad based spectrum of activity as it is effective not only on ticks such as *Ixodes ricinus* and *Hylomma marginatum rufipes* but also potent on nymphs adult ticks (*Rhipicephalus appendiculatus*).

Tick repellence bioassay: The repellence results of *C. citratus*, *T. diversifolia* and *L. angustifolia* essential oil against adults of *R. appendiculatus* achieved in this study are presented in Fig. 1. High percentage repellence (100%) against adults of *R. appendiculatus* was recorded after 10 min interval when all the three essential oils were used. A hundred percent repellence in all the three concentrations (5, 10 and 15% v/v) used was also recorded. In general, the repellence strength of *T. diversifolia* essential oil was not as strong compared to essential oils of *C. citratus* and *L. angustifolia*, particularly when 5% v/v concentration was used. EC_{50}, ED_{75} and ED_{95} generally increased with increasing time for all the three essential oils used (Fig. 2).

Toxicity bioassay: The toxicity results of *C. citratus*, *T. diversifolia* and *L. angustifolia* essential oils on *Rhipicephalus appendiculatus*, nymph and adult ticks at different time periods and their survival is presented in Fig. 2. Behavioural change was observed in the nymph in the first 20 min of exposure to

TABLE-4
CHEMICAL COMPOSITION OF *Lavandula angustifolia*

No.	Name of compound	m.f.	m.w.	Retention times	Peak areas (%)
1	α-Pinene	$C_{10}H_{16}$	136.34	935	0.09
2	Camphene	$C_{10}H_{16}$	136.23	951	0.23
3	Sabinene	$C_{10}H_{16}$	136.23	974	0.04
4	1-Octen-3-ol	$C_8H_{16}O$	128.21	995	0.53
5	β-Myrcene	$C_{10}H_{16}$	136.23	998	0.55
6	Delta-3-carene	$C_{10}H_{16}$	136.34	1031	0.14
7	*p*-Cymene	$C_{10}H_{14}$	134.21	1025	0.09
8	Limonene	$C_{10}H_{16}$	136.23	1029	0.55
9	*trans*-Sabinene hydrate	$C_{10}H_{18}O$	154.24	1098	1.40
10	*trans*-β-Ocimene	$C_{10}H_{16}$	136.23	1050	0.35
11	Linalool oxide	$C_{10}H_{18}O_2$	170.25	1087	0.30
12	*trans*-Linalool oxide	$C_{12}H_{20}O_3$	212.28	1072	0.24
13	Linalool	$C_{10}H_{18}O$	154.25	1097	29.7
14	1-Octen-3-yl acetate	$C_{10}H_{18}O_2$	170.25	1106	2.80
15	Lavandulol	$C_{10}H_{18}O$	154.25	1162	1.70
16	Borneol	$C_{10}H_{18}O$	154.25	1165	0.85
17	Terpinen-4-ol	$C_{10}H_{18}O$	154.25	1179	0.56
18	α-Terpineol	$C_{10}H_{18}O$	154.25	1189	4.35
19	Nerol	$C_{10}H_{18}O$	154.25	1230	0.65
20	Linalyl acetate	$C_{12}H_{20}O_2$	196.29	1257	47.56
21	(*E*)-Citral	$C_{10}H_{16}O$	152.23	1341	0.17
22	*p*-Cymen-8-ol	$C_{10}H_{14}O$	150.22	1183	0.06
23	Neryl acetate	$C_{12}H_{20}O_2$	196.29	1356	1.15
24	Geranyl acetate	$C_{12}H_{20}O_2$	196.29	1373	1.95
25	α-Santalene	$C_{15}H_{24}$	204.35	1418	0.62
26	*trans*-Caryophyllene	$C_{15}H_{24}$	204.35	1419	3.76
27	α-Bergamotene	$C_{15}H_{24}$	204.35	1435	0.19
28	(*E*)-β-Farnesene	$C_{15}H_{24}$	204.35	1455	0.53
29	α-Humulene	$C_{15}H_{24}$	204.35	1485	0.12
30	Germacrene-D	$C_{15}H_{24}$	204.35	1486	0.13
31	Caryophyllene oxide	$C_{15}H_{24}O$	220.35	1582	1.29
32	epi-α-Cadinol	$C_{15}H_{26}O$	222.37	1638	0.10

Key
High relative amount compound with no anti-tick properties
High relative amount compound with anti-tick properties
Low relative amount compound with anti-tick properties

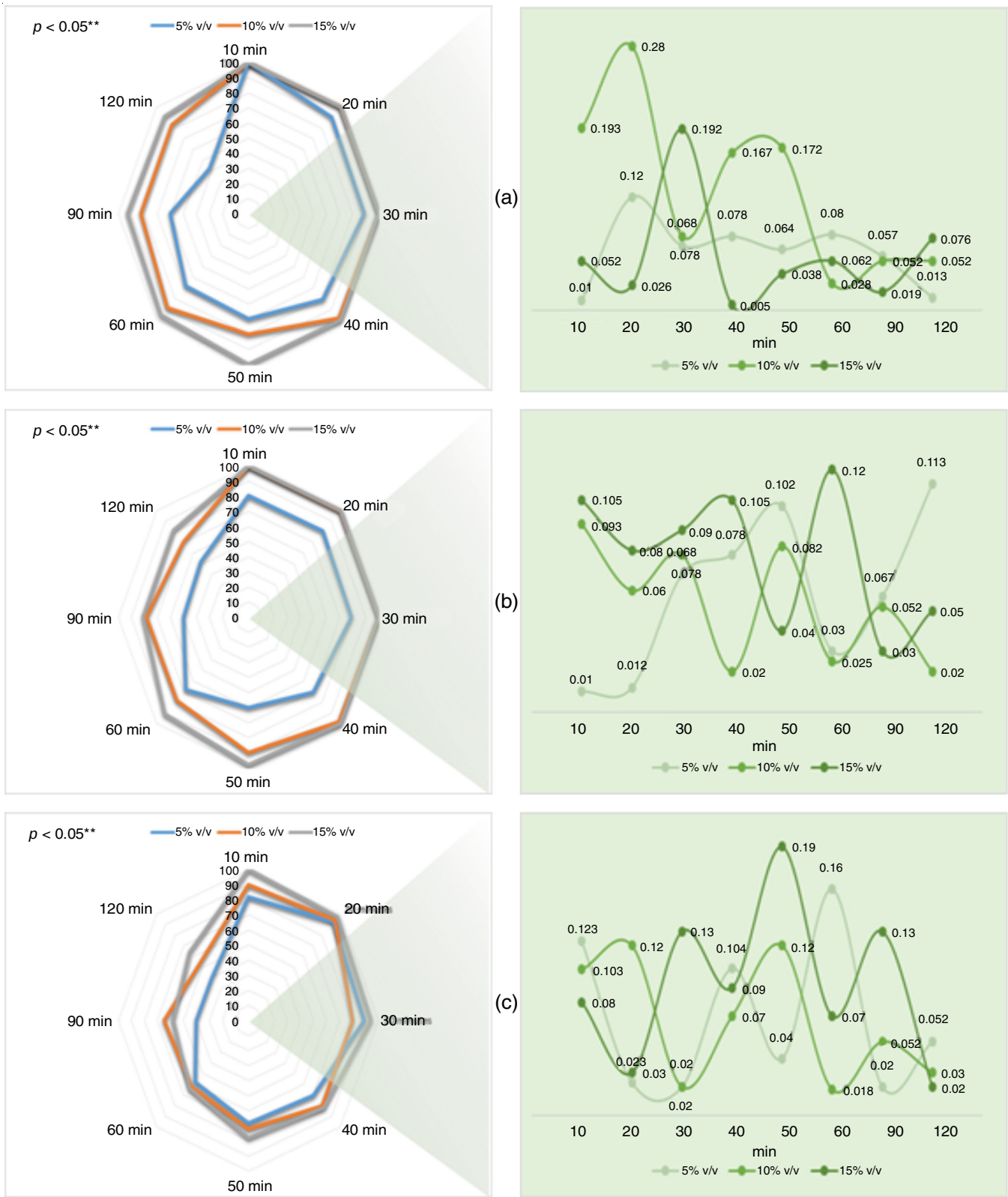

Fig. 1. Mean percentage repellency of (a) *C. citratus*, (b) *T. diversifolia* and (c) *L. angustifolia* essential oil against adults of *R. appendiculatus*. $p < 0.05$: for each time interval there was significance difference between the treatment and control. EC = Effective concentration to repell ticks

C. citratus, *T. diversifolia* and *L. angustifolia* essential oils at the concentration of 15% v/v. Although mortality was observed after 30 min with 50% mortality (LC_{50}) recorded for the nymph (Fig. 2a), a different result was obtained when adults ticks were used. When *C. citratus* was used, mortality started after 30 min and 50% mortality (LC_{50}) was reached after 24 h when 5 and

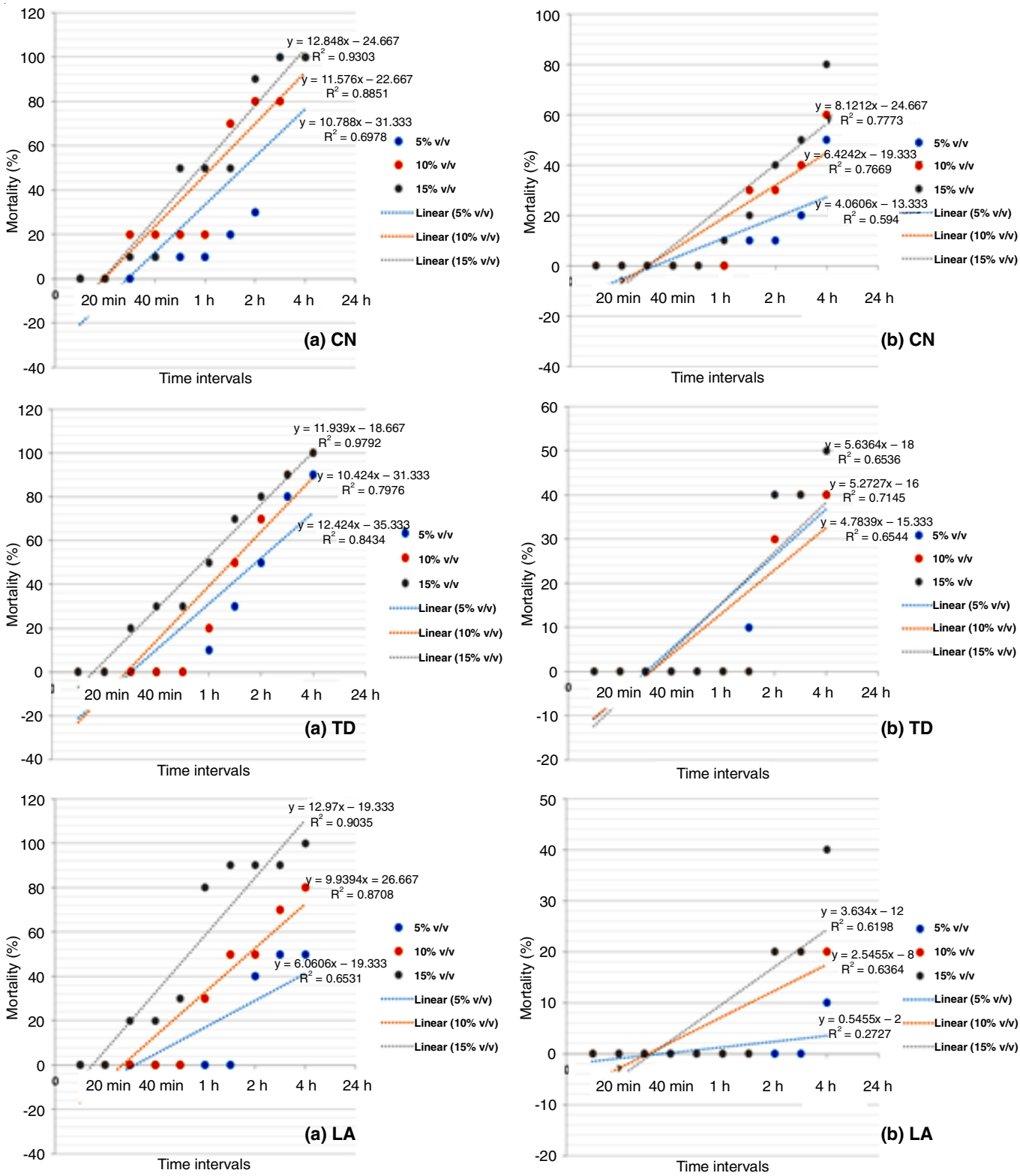

Fig. 2. Mortality of *R. appendiculatus* (a) nymphs and (b) adults ticks exposed to (CN) *C. citratus*, (TD) *T. diversifolia* and (LA) *L. angustifolia* essential oils over 24 h

10% v/v concentrations was used (Fig. 2b). However, when *T. diversifolia* essential oil was used, 50% mortality was reached after 24 h only when 15% v/v concentration was used. *L. angustifolia* did not reach 50% mortality even after 24 h of exposure. The lethal concentrations LC_{50} and LC_{95} for *C. citratus* were estimated to be 5.7%; 3.05% v/v and 10.9%; 9.09% v/v after 24 h exposure of adult; nymph ticks, respectively (Fig. 2). Also the LC_{50} and LC_{95} for *T. diversifolia* were estimated to be 14.02%; 5.56% v/v and 22.04%; 13.08% v/v after 24 h exposure of adult; nymph ticks, respectively. *L. angustifolia* showed LC_{50}

and LC_{95} of 19.98%; 5.08% v/v and 30.05%; 15.01% v/v after 24 h exposure of adult; nymph ticks, respectively. Statistical testing showed that at 15% v/v concentration a significant ($p < 0.05$) toxic effect on *R. appendiculatus* was achieved within 24 h when compared with initial time intervals for *C. citratus* ($R^2 = 0.9303$ nymphs; $R^2 = 0.7773$ nymphs), *T. diversifolia* ($R^2 = 0.9792$ nymphs; $R^2 = 0.6536$) and *L. angustifolia* ($R^2 = 0.9035$ nymphs; $R^2 = 0.6198$) essential oils used. Although this study showed the plant induced mortality against *R. appendiculatus* nymphs and adults ticks, the repellence strength of *T. diversifolia* essential oil was not so strong compared to essential oils of *C. citratus* and *L. angustifolia*, particularly when 5% v/v concentration was used. This may be due to the observed change of colour of the formulated essential oil of *T. diversifolia*.

Moulting inhibition bioassay: The essential oil of *C. citratus*, *T. diversifolia* and *L. angustifolia* completely inhibited the moulting ability of the engorged larvae of *Rhipicephalus appendiculatus* to zero percentage moulting.

Conclusion

The control of ticks is a key step for the growth of livestock farming. As a result, farmers in rural communities have continued to take alternative measures for the control and prevention of tick with plants of ethno-veterinary use. The study utilized the essential oils of three plants, which was selected after an ethnobotanical survey was conducted in the Ermelo community, Mpumalanga province in South Africa. Although several factors including environmental conditions, time of harvest and age of plant have been shown to affect the phytochemical profiles and the major chemical constituents of the studied essential oils. The essential oils of *C. citratus*, *T. diversifolia* and *L. angustifolia* showed the presence of the useful constitutents exhibiting the anti-tick activity. All the essential oils to variable extent demonstrated acaricidal and repellence activity against *R. appendiculatus* and also the method of extraction may have influenced the potency of the essential oils. The study has validated the use of plants by farmers for the control of ticks. Further investigation is on-going to isolate the major active chemical compounds of the oils and to evaluate different formulation ratios for synergistic or additive acaricidal and repellence activity against *R. appendiculatus*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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