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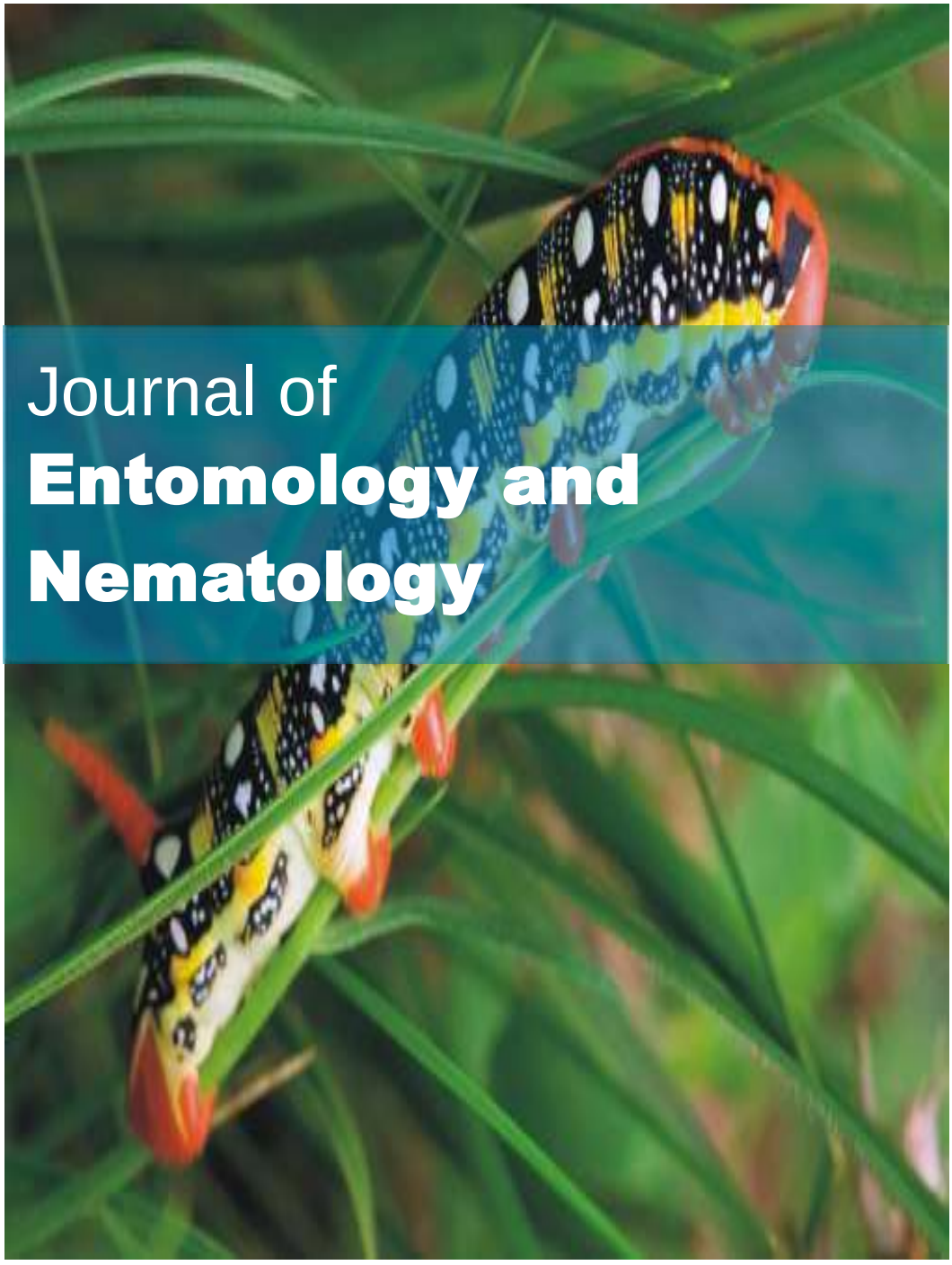

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Journal of Entomology and Nematology

Full Length Research Paper

Acaricidal effect of essential oil of *Clausena anisata* (Rutaceae) on larvae of three tick species: *Amblyomma variegatum, Rhipicephalus (Boophilus) decoloratus* and *Rhipicephalus (Boophilus) microplus*

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Control measures of livestock ticks based on chemicals has become worrisome because of the development of resistance, especially in the case of *Rhipicephalus (Boophilus) microplus*. It has become imperative to search for alternative tick control methods. Thus, the toxicity of the essential oil of *Clausena anisata* was evaluated on larvae of *Amblyomma variegatum*, *Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus* (*Boophilus*) *decoloratus* in the laboratory in comparison with flumethrin, a reference chemical acaricide. Tests were carried out with Whatman paper circles impregnated with 200 µL of the essential oil of *C. anisata* either pure or diluted with palm kernel oil (v/v) at concentrations of 0.0625, 0.05, 0.042, 0.03125, 0.025, 0.020, 0.0156, 0.0125 and 0.01. A total of 100 larvae aged 15-21 days were tested with the respective doses on filter paper in Petri dishes. The Petri dishes were incubated at 28 ± 1°C, 85-95% RH and mortalities were recorded after 24 h. Chemical composition of leaf essential oil of *C. anisata* major compounds were estragol (57.06%) and trans-anethole (29.88%), constituting 86.94% of the crude extract. Dilution of 0.0625 caused 100% larval mortality in the three species. The mortalities were similar for *A. variegatum* and *Rh. (B.) decoloratus* at the LD_{50} (0.021). The LD_{99} of *R. (B.) decoloratus* was the lowest (0.075). Flumethrin was very toxic with 100% mortality for *A. variegatum* even at the lowest dose (0.01), unlike species of the genus, *Rhipicephalus (B.)* which were less sensitive. These results on the use of *C. anasata* as bio-acaricide and further studies will provide a perspective in response to the resistance of ticks to chemical acaricides.

Key words: Cattle, ticks, larvae, *Clausena anisata*, Modified Larval Packet Test (MLPT), bio-acaricide.

INTRODUCTION

Livestock ticks cause heavy economic losses to breeders through reduced productivity, leather recovery and the diseases they transmit (Walker, 2014; Farougou et al.,2013). Recent studies in West Africa have revealed the introduction of new tick species including *Rhipicephalus (Boophilus) microplus* which is spreading rapidly in the sub-region (De Clerq et al., 2015; Adakal et al., 2013). In Togo, this species was reported in

association with *Amblyomma variegatum* and *Rhipicephalus (B.) decoloratus* (Mollong et al., 2018) as characteristic species in its Maritime Region. The repeated application, use of the wrong dosage and the lack of mastery of synthetic acaricide use techniques in the control of these ectoparasites have led to the development of resistance to a growing number of their molecules (De Meneghi et al., 2016; Adakal et al., 2012). Currently, *Rhipicephalus (B.) microplus* has become resistant to several molecules belonging to various classes of chemical acaricides (Adehan et al., 2016; Abbas et al., 2014). This tick, which is able to displace other species of the same genus, has spread to several African sub-regions at present (Boka et al., 2017; Biguezoton et al., 2016; Boka et al., 2014; Madder et al., 2012). Also, its synergistic action with other species represents a real danger for cattle breeding and requires prophylactic measures to keep the situation under control. It is therefore necessary to exploit new control alternatives. Several authors (Yessinou et al., 2016; Chagas et al., 2014) have shown that some plant essential oils have acaricidal properties, although these essential oils are volatile. These bio-acaricides, because of the many constituents, and their multiple modes of action, the appearance of resistance in ticks is reduced unlike chemical acaricides (Villaverde et al., 2016). Their availability and potential long-term use is an advantage. These observations motivated the present research on the essential oil of *Clausena anisata* (Willd). Hook in the perspective of its use for tick control. Specifically, the acaricidal activity of this essential oil on the larvae of three tick species and the capacity of palm kernel oil used as solvent, to retain the acaricidal constituents in the essential oil, were studied.

MATERIALS AND METHODS

Study area

This study was carried out at Agricultural Experiment Station of the Graduate School of Agronomy, University of Lomé. The university lies between AES-GSA-UL: 06°10´48.6´´N, 001°12´66.1´´E and 35 m. The station has a Guinean tropical climate with particularly rainfall of 800 to 1000 mm/year, temperatures ranging from 25-31°C and average relative humidity of between 74 and 90%. The sunshine is 6 h/day and the photoperiod is 12L: 12D.

Tested products

Essential oil of the leaves

Essential oil of the leaves of *C. anisata* was provided by the Laboratory of Natural Extracts and Aromas (LEVAN) of the University of Lomé (Togo). This oil was analyzed by gas chromatography coupled with mass spectrometry to identify its major constituents. The analysis was carried out by Sarl Pyrenessences Analyses-2, chemin de la plaine-11340 Belcaire in France.

Palm kernel vegetable oil

The virgin palm kernel vegetable oil was purchased from a supplier who obtained it by squeezing the crushed and steamed seeds for 15 to 20 min.

Flumethrin

The chemical acaricide used was FLUMAX manufactured by ASHISH LIFE SCIENCE PVT LIMITED 213, Laxmi Plaza, New Link Road, Andheri (W), Mumbai-53, India. It is composed of flumethrin 1% (m/v) and belongs to the class of synthetic pyrethroids. It was used as a reference acaricide to assess the toxicity of the essential oil of *C. anisata*.

Collection and preparation of engorged female ticks for egg laying

The engorged females of the different species were collected from cattle on the Agricultural Experiment Station of the Graduate School of Agronomy of the University of Lomé between 6 am and 8 am before the animals left for grazing. After restrainting each animal, “tire-tique” was used to remove the ticks. For good aeration, boxes with 1 mm diameter perforations were used to transport ticks to the Laboratory of Applied Entomology (LAE). The ticks were first washed with distilled water and wiped with towel paper and then thoroughly examined to ensure that they are in good condition for egg laying. Morpho-anatomical identification key of Ixodidae by Walker et al. (2014) was used to identify ticks. A very sensitive balance (SARTORIUS GMBH GÖTTINGEN type PT120) was used to weigh them. Engorged females of *A. variegatum* that were kept for egg-laying, weighed between 2000-4000 mg, and those of *R. (B) decoloratus* and *R. (B) microplus* weighed between 150-350 mg. The eggs were collected every 3 or 4 days of laying, mixed, put in new boxes and incubated in the same rearing room for the laying females at 28 ± 1°C, 80-95% relative humidity (RH) and 12:12 L:D approsimative photoperiod. These conditions were checked every day by means of a thermo-hygrometer (type: TROTEC BZ05) to monitor the environment until hatching. A binocular loupe (type: LEICA EZ4) was used to check the status of eggs and larval mortality.

Dilutions and preparation of Petri dishes for Modified Larval Packet Test (MLPT)

Test dilutions

Serial dilutions of the crude extract of *C. anisata* essential oil in virgin palm kernel vegetable oil (v/v) were made in order to obtain mixtures with concentrations of 0.0625, 0.05, 0.042, 0.03125, 0.025, 0.020, 0.0156, 0.0125 and 0.010. Flumethrin dilutions were

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made in the same way.

Preparation of Petri dishes and toxicity test

Glass Petri dishes of 8 cm diameter and 1 cm height we used. The bottom of each Petri dish was upholstered with a Whatman filter paper (Grade 1: 460 mm × 570 mm; CAT N°1001-917) 8 cm in diameter. Using a Humapette micropipette, 200 µL of each dilution was deposited in small drops on Whatman paper surface in each Petri dish which was immediately closed. After about 15 min, the mixture spreads homogeneously over the entire surface of the filter paper. The impregnation of the Whatman filter paper is made from the lowest dose to the strongest to avoid contamination. One hundred (100) 15-21 days old larvae were deposited the same day 15 min after the mixture spread homogeneously in the middle of the Whatman filter paper and immediately covered by second filter paper in each Petri dish which is sealed and incubated at 28 ± 1°C, 80-95% RH and 12L: 12D. The negative control Whatman filter paper was impregnated solely with palm kernel vegetable oil. The positive controls were impregnated with the crude extract of *C. anisata* essential oil or 1% flumethrin. Five replicates were made and larval mortality was assessed after 24 h of exposure. Concentrations of the essential oil of *C. anisata* and the reference acaricide in the mixtures were determined by considering their volumes in the 200 µL (V/V).

Evaluation of the afterglow of the essential oil

Afterglow is the persistence of the toxic effect of a product tested on a support or in a given environment. To evaluate the persistence of the essential oil, the crude extract and the dilution of 0.0625% in palm kernel oil were tested on the larvae of *R. (B) microplus*. For each product, a series of nine (9) Petri dishes was prepared. Each Whatman filter paper of the first series was impregnated with 200 µL of crude oil extract and each Whatman filter paper of the second series was impregnated with 200 µL of the 0.0625% dilution. Immediately after homogeneous spreading, all dishes were opened and left open under ambient laboratory conditions: 26 - 30°C, 54 - 80% RH and 12:12 L:D so that in each series, the essential oil could evaporate during nine specific periods of time; 0 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 12 h and 24 h. After these time periods, one hundred (100) 15 to 21 days old larvae were deposited in the middle of the impregnated Whatman filter paper in each dish before the Petri dish was sealed and incubated at 28 ± 1°C and 80-95% RH and 12:12 L:D. Five replicates were made for each product and time interval and larval mortality was assessed after 24 h of exposure.

Mortality assessment

Natural mortality of larvae species was determined in the controls using:

$$\text{Control mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

To calculate the average mortality at the different doses, the following formula was applied:

$$\text{Average mortality (\%)} = \frac{\text{Mortality 1} + \text{Mortality 2} + \text{Mortality n}}{\Sigma ni}$$

Where, n = number of replicate; Mortality 1 = number of dead larvae in the first replicate; ni = total number of larvae tested in each replicate

In tests where control mortalities exceeded 5% or more, Abbott's (1925) formula for calculating corrected mortality was applied:

$$\text{Corrected mortality (\%)} = \frac{\text{\% Mortality in the test - \% Corrected mortality}}{\text{100\% - \% Corrected mortality}} \times 100$$

Data analysis

Statistical treatment of mortality data was done using a 5% analysis of variance (ANOVA) with SPSS.v.16.0 software. Mortality averages were discriminated with the least significant difference (LSD) test, using the same software. Nonlinear regression analyses of the dose-mortality data were performed with the Polo Plus software (version 1.0) using the Dose-Response Curves Probit and Logit Analyses. Values for 95% LD_{50} and LD_{99} and their confidence intervals (95% CI) and resistance ratios (RRs) were estimated by probit analysis (Polo plus version1 software).

RESULTS

Composition of the essential oil of the leaves of *C. anisata*

The analysis of this essential oil showed that estragol (57.06%) and trans-anethole (29.88%) were the major compounds, constituting 86.94% of crude extract. Other compounds exceeding 1% are anisaldehyde (2.67%), p-cymene (2.31%) and α-pinene (1.07%) (Table 1).

Mortality of larvae aged 15 to 21 days

Effects of crude products

Only the crude essential oil of *C. anisata* and flumethrin produced 100% mortality in the larvae. Palm kernel oil had no effect on the larvae which in these experimental conditions survived during an average of three months for *A. variegatum* and two months for *R. (B) decoloratus* and *R. (B) microplus* (Table 2).

Toxicity of different dilutions

The tests with the different dilutions of products on the

Table 1. Chemical composition of leaf essential oil from *C. anisata.*

Peak	Retention time (min)	Chemical constituents	Composition (%)
1	7.7	α-Pinene	1.07
2	10.0	β-Pinene	0.17
3	11.6	β-Myrcene	0.25
4	12.8	Limonene	0.29
5	14.3	γ-Terpinene	0.23
6	15.2	p-Cymene	2.31
7	21.6	Citronellal	0.14
8	23.3	Linalool	0.14
9	25.2	β- caryophyllene	0.22
10	27.1	Estragol	57.06
11	27.3	α-Humulene	0.20
12	28.4	Z- β-Farnesene	0.32
13	28.5	Neral	0.34
14	29.0	Citronellol	0.19
15	29.1	Cis-Anethole	0.10
16	29.6	α-Curcumene	0.22
17	31.0	Trans-Anethole	29.88
18	34.8	Caryophyllene oxide	0.40
19	35.6	Anisaldehyde	2.67
20	36.1	Epoxy-6,7- Humulene	0.23
21	38.2	Anicetone	0.19
22	46.4	3-Methoxycinnamaldehyde	0.32
23	49.7	Hydroxyphenyl butanone Mw=164	0.30
		Total	97.24

Table 2. Average mortalities (% ± SD) and survival time of larvae aged 15-21 days at 28 ± 1 ° C, 80-95% RH and 12L: 12D treated with different products. (n = 100).

Crude products tested	Tick species	Mortality (% ± SD)*	Survival time (days ± SD)*	Statistical test
Palm kernel oil	*A. variegatum*	0	95.4 ± 9.23^{a}	$F_{(14, error)} = 27.382$; P<0.0001
	R. (B) decoloratus	0	53± 10.74^{b}	
	R. (B) microplus	0	60.2 ± 9.01^{b}	
C. anisata essential oil	*A. variegatum*	100	-	
	R. (B.) decoloratus	100	-	
	R. (B.) microplus	100	-	
Flumethrin (1%)	*A. variegatum*	100	-	-
	R. (B.) decoloratus	100	-	
	R. (B.) microplus	100	-	

*Averages with the same letter in the same product tested are not significantly different (ANOVA followed LSD test, P <0.05).

larvae of the different tick species showed that larval mortality was dose-dependent. The 1/16 dilution (0.0625) of *C. anisata* essential oil resulted in 100% mortality of *A. variegatum* and *R. (B.) decoloratus* larvae, as well as very high mortality (99.8 ± 0.44) for *R. (B.) microplus*. Larval mortality at the same dilution with flumethrin was 100% for *A. variegatum*, 77.6 ± 7.09 for *R. (B.) decoloratus* and only 42.6 ± 5.02 for *R. (B.) microplus* (F (29, error) = 216.083, P <0.0001) (Table 3).

A. variegatum was therefore more sensitive to the two products tested than *R. (B.) microplus* which is the least sensitive species to the two products tested. Also, the

Table 3. Average mortalities (**% ± SD)** of larvae aged 15 to 21 days with different dilutions at 28 ± 1°C, 80 - 95% RH and 12L: 12D. (n = 100).

Products tested	Tick species	Dilutions (V/V) of the essential oil of *C. anisata* and flumethrin									Statistical test
		(1/16)	(1/20)	(1/24)	(1/32)	(1/40)	(1/48)	(1/64)	(1/80)	(1/96)	
C. anisata	*A. variegatum*	100 ± 00^{aA}	97 ± 2.73^{aA}	82.4 ± 6.06^{bBC}	66 ± 5^{cBC}	54.6 ± 4.61^{dBC}	43.4 ± 5.54^{eB}	38.2 ± 7.25^{eB}	22.8 ± 3.34^{fB}	9.8 ± 3.42^{gB}	F = 231.757 Df = 44 ; P<0.000
	R. (B.) decoloratus	100 ± 00^{aA}	94.6 ± 3.20^{bA}	87.8 ± 3.27^{cB}	69.6 ± 2.30^{dB}	58.8 ± 4.08^{eB}	49.6 ± 8.01^{fB}	30.8 ± 3.19^{gC}	13.2 ± 5.06^{hC}	8.4 ± 1.94^{hB}	F = 359.509 Df = 44; P<0.000
	R. (B.) microplus	99.8 ± 0.44^{aA}	88.8 ± 4.96^{bB}	80.6 ± 3.36^{cC}	61.4 ± 3.64^{dC}	52 ± 3.39^{eC}	35.6 ± 5.5^{fC}	16.4 ± 6.46^{gD}	5.6 ± 2.06^{hD}	0.8± 0.83^{hC}	F = 438.885 Df = 44; P<0.000
Flumethrin	*A. variegatum*	100 ± 00^{aA}	100 ± 00^{aA}	100 ± 00^{aA}	100 ± 00^{aA}	100 ± 00^{aA}	100 ± 00^{aA}	100 ± 00^{aA}	100 ± 00^{aA}	100 ± 00^{aA}	F = 0 Df = 44; P= 0
	R. (B.) decoloratus	77.6 ± 7.09^{aB}	59.8 ± 3.70^{bC}	48.2 ± 5.06^{cD}	35.6 ± 5.98^{dD}	25 ± 3.16^{eD}	19.8 ± 2.77^{eD}	12.6± 2.70^{fD}	5.6 ± 3.36^{gD}	0 ± 00^{hC}	F = 188.467 Df = 44; P<0.000
	R. (B.) microplus	42.6 ± 5.02^{aC}	37.2 ± 4.81^{bD}	25.6 ± 4.56^{cE}	12.8 ± 3.03^{dE}	8.2 ± 2.94^{eE}	6.6 ± 2.6^{efE}	2.4 ± 2.3^{fgE}	0 ± 00^{gE}	0 ± 00^{gC}	F = 118.807 Df = 44; P<0.000
Statistical test		F = 216.083 Df = 29 P<0.000	F = 243.201 Df = 29 P<0.000	F = 223.706 Df = 29 P<0.000	F = 305.945 Df = 29 P<0.000	F = 437.640 Df = 29 P<0.000	F = 223.410 Df = 29 P<0.000	F = 314.476 Df = 29 P<0.000	F = 816.628 Df = 29 P<0.000	F = 2.892E3 Df = 29 P<0.000	-

*Averages with the same lowercase letter on the same row are not significantly different (ANOVA followed by LSD test, P <0.05); * Averages with the same capital letter in the same column are not significantly different (ANOVA followed by LSD test, P <0.05).

1/40 dilution (0.025) of *C. anisata* essential oil caused larval mortality greater than 50% in all species with averages of 54.6 ± 4.61, 58.8 ± 4.08 and 52 ± 3.39, respectively, on *A. variegatum, R. (B.) decoloratus* and *R. (B.) microplus*, while flumethrin resulted in 100% mortality on *A. variegatum* (F (29, error) = 437.640, P <0.0001). Flumethrin was highly toxic to *A. variegatum* with 100% larval mortality at all dilutions, unlike the other two species, especially *R. (B.) microplus*, which was less sensitive to flumethrin than essential oil of *C. anisata* for all dilutions tested (Table 3).

Dose-response curves for essential oil dilutions of *C. anisata* (Figure 1) generally showed the same mortality trend for the three species. Particularly, perfect similarity was observed between the dose-response curves of *A. variegatum* and *R. (B.) decoloratus*, while the dose-response curve of *R. (B.) microplus* remain distinct from the two previous curves showing lower mortalities as compared to the other two species. This trend was confirmed by LD_{50} of *A. variegatum* and *R. (B.) decoloratus* (0.021/0.021). In contrast, LD_{50} of *R. (B.) microplus* was slightly higher (0.026). The LD_{99} of *R. (B.) decoloratus* was the lowest (0.075), whereas that of *A. variegatum* was the highest (0.087) (Table 4). (RR) CIs were low, showing that all the species were susceptible to *C. anisata* essential oil. Dose-response curves for dilutions of flumethrin (Figure 2) shows totally divergent mortality trends between the three species. For *A. variegatum* flumethrin was highly toxic with 100% mortality at the lowest dose (0.01). In contrast, larval mortality was dose-dependent for the other species. *R. (B.) decoloratus* was more sensitive to flumethrin than *R. (B.) microplus*. The LD_{50} and LD_{99} of these species which are respectively 0.040/0.062 and 0.208/0.276 (Table 5) confirm this. *R. (B.) microplus* was less sensitive to flumethrin. There was also no overlap between the confidence intervals (CIs) of the different LDs for each species; there is therefore a significant difference between the LDs of each species. The CIs of resistance ratios (RRs) for *R. (B.) microplus* were low; this shows that although mortality is low, most individuals of these species have been

Table 4. Lethal doses (LD_{50} and DL_{99}) and *C. anisata* resistance ratio (LD_{50} and DL_{99}).

Species	*C. anisata*				Slopes
	LD_{50} (IC)	LD_{99} (IC)	RR_{50}	RR_{99}	
A. variegatum	0.021(0.019 ± 0.024)	0.087(0.067 ± 0.130)	-	-	3.797±0.100
R. (B.) decoloratus	0.021 (0.020 ± 0.023)	0.075(0.064 ± 0.091)	0.987(0.952 ± 1.024)	1.159(1.048 ± 1.281)	4.282±0.108
R. (B.) microplus	0.026 (0.024 ± 0.028)	0.084(0.072 ± 0.104)	0.815(0.786 ± 0.845)	1.030(0.929 ± 1.143)	4.553±0.124

LD = 95% lethal dose; IC = 95% confidence interval; RR = resistance ratio.

Table 5. Lethal Doses (LD_{50} and DL_{99}) and flumethrin Resistance Ratio (LD_{50} and DL_{99}).

Species	Flumethrin				Slopes
	LD_{50} (IC)	LD_{99} (IC)	RR_{50}	RR_{99}	
A.variegatum	-	-	-	-	54.633± 4667207.699
R. (B.)decoloratus	0.040(0.037 ± 0.044)	0.208(0.159 ± 0.300)	-	-	3.239±0.099
R. (B.) microplus	0.062(0.055 ± 0.074)	0.276(0.194 ± 0.468)	0.642(0.592 ± 0.695)	0.754(0.590 ± 0.963)	3.589±0.178

LD = 95% lethal dose; IC = 95% confidence interval; RR = resistance ratio.

Table 6. Average mortalities of larvae aged 15 to 21 days with crude essential oil of *C. anisata* and at dilution of LD_{99} (1/16) at 28 ± 1°C, 80 - 95% RH and 12L : 12D (n = 100).

Time between introduction of larvae in boxes	Mortality (% ± SD)*		Statistical test
	C. anisata pure	DL_{99} (1/16)	
0 h	100 ± 00^{aA}	99.8 ± 0.44^{aA}	$F_{(89, error)} = 413.142$; P< 0.0001
1/2 h	100 ± 00^{aA}	78 ± 6.70^{bB}	
1 h	100 ± 00^{aA}	67.6 ± 4.27^{bC}	
2 h	100 ± 00^{aA}	60.8 ± 4.49^{bD}	
3 h	100 ± 00^{aA}	53.4 ± 4.66^{bE}	
4 h	100 ± 00^{aA}	51.2 ± 3.56^{bE}	
6 h	100 ± 00^{aA}	43.2 ± 3.42^{bF}	
12 h	97 ± 3.46^{aA}	33 ± 4.74^{bG}	
24 h	13.2 ± 3.70^{bB}	26.2 ± 4.81^{aH}	
Statistical test	$F_{(44, error)} = 1.455^{E}3$; P< 0.0001	$F_{(44, error)} = 133.3$; P< 0.0001	

h = hour; *Averages with the same small letter on the same row are not significantly different (ANOVA followed by LSD test, P <0.05)
*Averages with the same capital letter in the same column are not significantly different (ANOVA followed by LSD test, P <0.05).

sensitive to flumethrin.

Remanence of the crude extract of C. anisata essential oil and its 1/16 dilution in R. (B.) microplus species

Retention test of *C. anisata* crude essential oil and its 1/16 dilution in palm kernel oil on Whatman paper was performed on *R. (B.) microplus,* a less sensitive species. The crude essential oil of *C. anisata* showed high toxicity during the first 12 h with an average larval mortality greater than 97 ± 3.46%. However, at 24 h, this mortalitydecreased to 13.2 ± 3.70% (Table 6).

The 1/16 dilution produced high mortality only at the beginning of the tests (between zero and thirty minutes) with 99.8 ± 0.44% larval mortality (Table 6 and Figure 3). After 30 min, effectiveness decreased progressively to 26.2 ± 4.81% at 24 h. However, this mortality after 24 h was significantly greater than that obtained with the crude extract of *C. anisata* (13.2% ± 3.70) F (89, error) = 413.142; P <0.001. So, in the long run, palm kernel oil

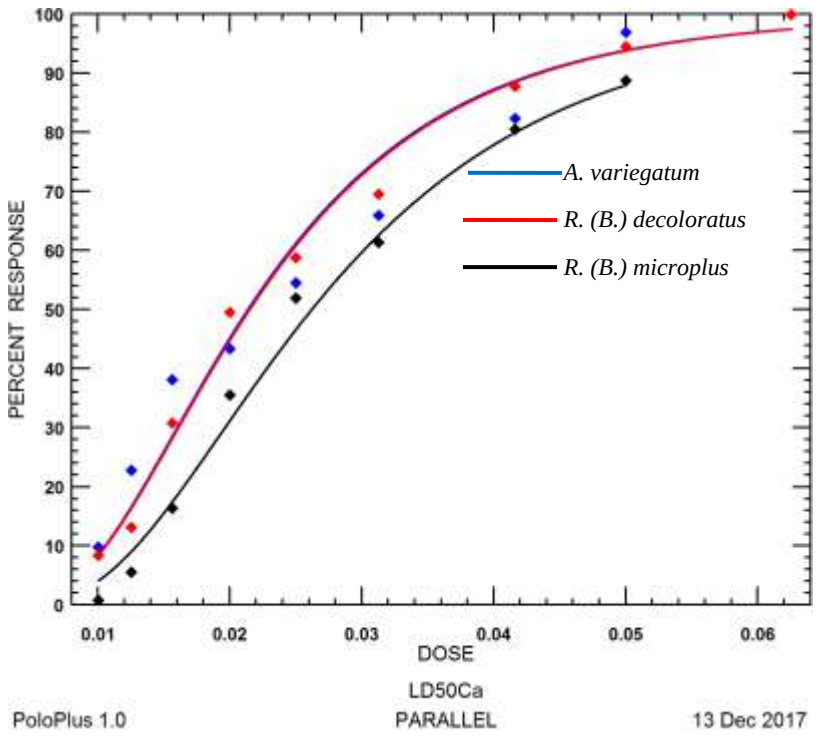

Figure 1. Dose-response curves of dilutions of *C. anisata* essential oil on the larvae of the three species.

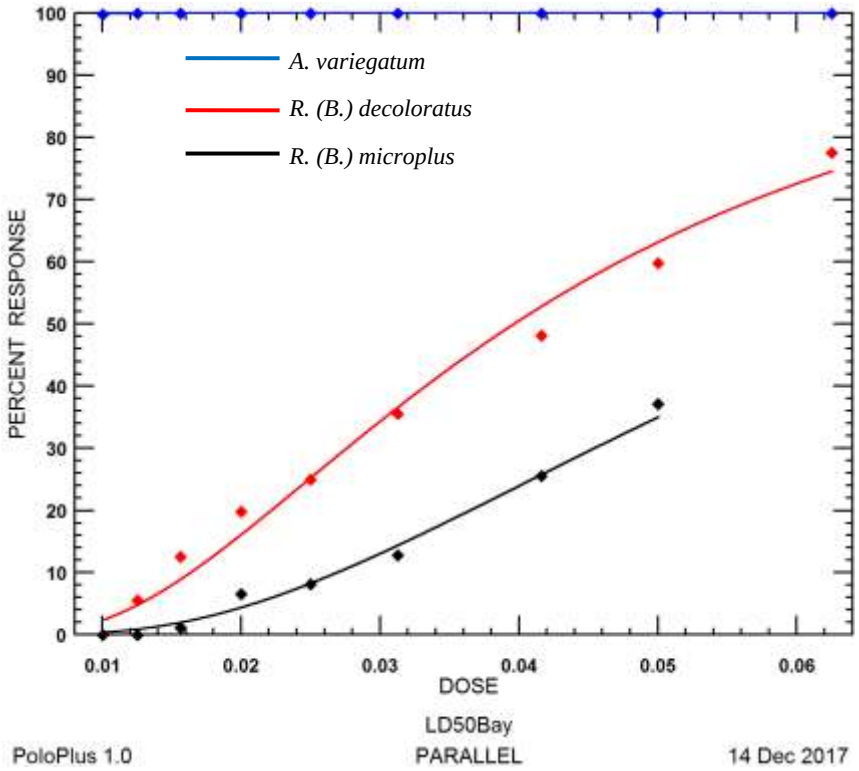

Figure 2. Dose-response curves for dilutions of flumethrin on the larvae of the three species.

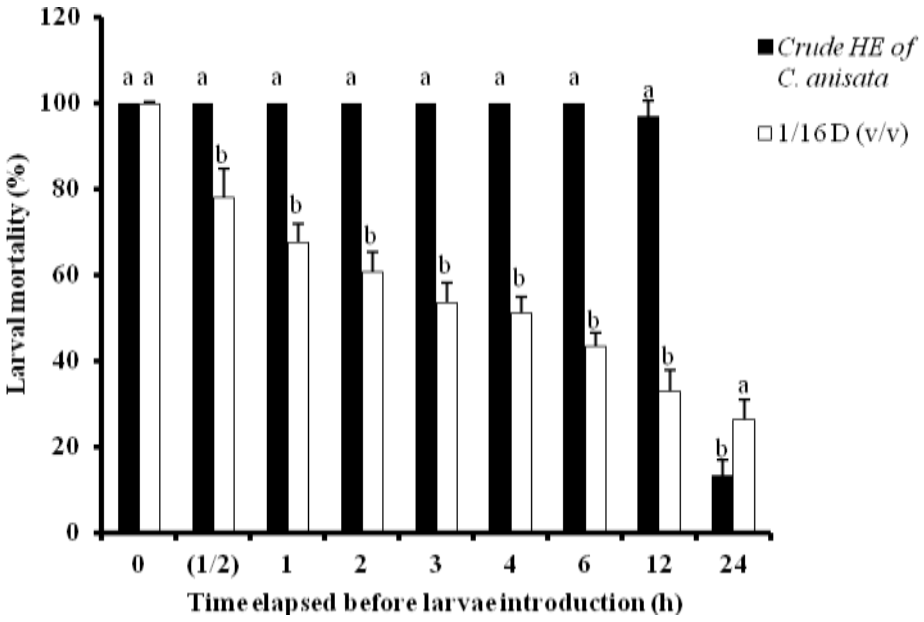

Figure 3. Variation in larval mortality of *R. (B.) microplus* treated with *C. anisata* crude essential oil and its 1/16 dilution with time at 28 ± 1°C, 80 - 95% RH and 12L : 12D

seems to favour the retention of the volatile essential oil.

DISCUSSION

One of fundamental problems is the question of the resistance that ticks develop to synthetic acaricides which also pollute the environment. Several authors (Yessinou et al., 2016; Silva et al., 2011, 2009; Nuto et al., 2008) have already tested the toxicity of essential oils on ticks and shown have that they constitute potential alternatives to chemical acaricides because of their effectiveness. However, they use different essential oil apart from *C. anisata* which, in addition to antifungal and microbial properties, is used as an ethnomedicinal plant (Lawal et al., 2015). Essential oil of *C. anisata* proved to be toxic to larvae of three species. Although, the mechanism of action of pesticidal properties is unknown and relatively under-researched (Isman 2000), Wigglesworth (1972) suggests that the lipophilic nature of essential oils can degrade the waxy layer of insects and cause water loss or larval desiccation. In these circumstances, the trachea and the air sacs of arthropods which are coated with this waxy layer, are affected by the essential oil and may lead to asphyxiation. The death of larvae obtained after exposure is certainly due to contact and/or inhalation of vapors of essential oil compounds. Essential oils have insecticidal activity and because of their volatility, are used as good fumigation materials (Amzouar et al., 2016). However, other authors (Chiasson et al., 2004a, b; Karpouhtsis et al., 1998) argued that, topical application or contact with the essential oils is necessary for mortality to occur. Nuto et al. (2008) and Alitonou et al. (2004) also confirmed this on ticks.

The observations in this study indicate that the vegetable oil of palm kernels used as solvent had no effect on the larvae of the species tested. Therefore, the larval mortality observed in this study is due either to the inhalation or to the contact of essential oil compounds with the larvae. Vinturelle et al. (2017) and Peixoto et al. (2015) have shown that the effectiveness of essential oils on these arthropods like *R. (B) microplus*, is due to the effect of some major compounds such as carvone, limonene and citral, β-aminoalcohol and other compounds. Also, regarding the essential oil of *C. anisata*, effectiveness of some major compounds like estragol has already been emphasized (Okunade and Olaifa, 1987). In fact, it is very easy for a pest to develop resistance against a molecule and in turn to molecules belonging to the same class as against a mixture of molecules (crude extract) acting synergistically (Torres et al., 2012; Chagas et al., 2011). These authors have already shown that the crude extract of essential oils is more effective on ticks than extracts derived or fractionated.

Stabilization of essential oils in an appropriate solvent will undoubtedly slow down its volatility and increase its effectiveness (Nuto et al., 2008). This hypothesis seems to be corroborated since it was found that exposure of *R. (B) microplus* larvae 24 h after impregnating Whatman paper with dilution of the essential oil in palm oil gave a higher mortality than the crude essential oil. The vegetable oil certainly retained some compounds that caused this mortality gap. However, this vegetable oil

retains very little essential oil since larval mortality dropped very rapidly after the thirtieth minute with a difference in mortality between the crude extract and the dilution. In addition, dilutions of the essential oil proved to be more effective than flumethrin with respect to species of the genus *Rhipicephalus (B)*, particularly *R. (B) microplus*. Flumethrin which proved highly toxic to *A. variegatum*, was less toxic to *R. (B) decoloratus* and especially *R. (B) microplus*. This is probably because these monophasic species spend their entire life cycle on the same host. This constantly exposes them to synthetic chemical acaricides and gives them a chance to develop more resistance to molecules (Adehan et al., 2016; Castro-Janer et al., 2010a, b). Thus, this resistance would predispose *R. (B.) microplus* to be less sensitive to other molecules which are prone to metabolization by similar enzymes or which are active on similar targets. The lower sensitivity of *R. (B.) microplus* larvae towards this essential oil and flumethrin in comparison with the other species endorses this fact. Essential oil of *C. anisata* is therefore toxic to larvae of the three tick species and constitutes an alternative to control their populations.

Conclusion

This study showed that essential oil of *C. anisata* is toxic to larvae of *A. variegatum, R. (B.) decoloratus* and *R. (B.) microplus* ticks. Toxicity is dose-dependent and a 1/16 dilution caused 100% mortality of larvae aged 15 to 21 days after 24 h of exposure. However, species sensitivity differs. In addition, palm kernel vegetable oil can reduce the volatility of the essential oil but only to a very small extent. Search for methods and means of application of this essential oil would be beneficial for its use in tick control.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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